

15 per cent lower than the total blood volume after the hematocrit reading has been restored to normal by the use of liver (10). It appears probable that a large part of this reduction of total volume is apparent rather than real, because after recovery the method gives a falsely high red cell volume, and hence a falsely high total volume. An hematocrit reading, nevertheless, is useful when taken in conjunction with the plasma volume determination because it is known that the plasma volume of otherwise normal individuals varies with the level of the hematocrit reading. It is probable that at rest the distribution of cells and plasma in the large and small vessels is relatively constant. This may account for the observation that the amount of blood removed from the body may be accurately quantitated by determining the plasma volume and hematocrit reading before and after the removal of a known quantity of blood, provided the post-hemorrhage determination is made before the hematocrit reading has fallen appreciably. If the post-hemorrhage determination is made after the hematocrit reading has decreased considerably, it is no longer possible to estimate accurately the amount of blood removed (9, 11).

It is not possible to measure accurately changes in plasma volume from changes in hematocrit reading or hemoglobin concentration, as this method is based on the assumption that there is an even distribution of red cells and plasma throughout the body. However, the direction of the change in plasma volume can be determined from the change in the hematocrit reading or hemoglobin concentration. Changes in hematocrit reading or hemoglobin concentration, which are not exactly proportionate to changes in plasma volume or protein concentration, do not mean that red cells have been added or removed from the general circulation. A shift in the proportion of blood present in the large and small vessels can produce changes of this nature. For example, calculation of the red cell volume from the plasma volume and hematocrit reading before and after strenuous exercise shows an apparent increase in the number of red cells in the circulation (12, 13). It seems probable that this increase is due to a difference in the distribution of red cells in the circulation because it occurs in splenectomized as well as in normal subjects (13).

#### SUMMARY AND CONCLUSIONS

1. A method for obtaining the blood contained in the minute vessels of the forearm is described. A distinction is drawn between blood flowing from the minute vessels and the blood contained within the minute vessels.
2. The venous blood is richer in cells and poorer in plasma than the blood contained within the minute vessels. Therefore, the cell plasma ratio of blood drawn from artery, vein, or finger is not representative of the cell plasma ratio of the entire circulating blood.
3. The value for the red cell volume, as calculated from the plasma volume and hematocrit reading, is falsely high because of the uneven distribution of cells. The value for the total circulating hemoglobin, as calculated on the basis of the plasma volume, hematocrit reading and hemoglobin concentration, is also falsely high.
4. It is not possible to quantitate accurately changes in plasma volume from the changes in hematocrit reading or hemoglobin concentration.

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# THE CLEARANCE OF BILIRUBIN FROM THE PLASMA. A MEASURE OF THE EXCRETING POWER OF THE LIVER

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The clearance of bilirubin from the plasma following intravenous injection was first used to measure the excretory function of the liver by Eilbott (1) and Von Bergmann (2) in Germany in 1927. In the United States the method was introduced by Harrop and Barron (3) in 1931. Additional experiences by Soffer (4, 5, 6, 7, 8) and by others (9, 10, 11, 12) have established the value of the test. Aside from the theoretical desirability of using a physiologic material (bilirubin) rather than a dye (bromsulphalein or rose bengal) to assess hepatic excretory function, it is generally agreed that the bilirubin test is unusually sensitive in demonstrating disturbed function when the degree of liver damage is relatively slight. The usefulness of the test and a desire to extend the range of clinical disorders to which it can be applied have prompted the present study.

As now used, the test involves determination of the percentage of injected bilirubin remaining in the circulation after a standard time period, usually 4 hours. Three samples of plasma are required. An initial sample *a* is obtained to determine the natural level of bilirubin in the patient's plasma. Pure bilirubin—1 mgm. per kilogram of body weight dissolved in a small volume of 0.1 M sodium carbonate solution—is injected intravenously. After allowing 4 to 5 minutes for mixing in the blood stream, a second sample of plasma *b* is secured. The final sample *c* is collected 4 hours later. If  $B_a$ ,  $B_b$  and  $B_c$  represent the bilirubin concentrations in each of the three plasma samples, the percentage retention equals  $100 (B_c - B_a) \div (B_b - B_a)$ . Reported results indicate that in many normal subjects there is no retention of pigment at the end of the 4-hour period. Soffer (7) considers retention in excess of 5 per cent to be evidence of impaired liver function.

It will be observed that the above method which utilizes percentage retention as the measure of hepatic function is based on the assumption that rate of excretion is not influenced by the basal level of bilirubin in the plasma. The value for this level is simply subtracted from each of the other levels before the retention is computed. It

will appear presently that the assumption is not justified. However, since most authors state that the test should not be applied when the basal level is above 1 mgm. per cent, and since the result is usually interpreted simply as normal or abnormal, and not as a quantitative measure of excretory function, it has in general served its purpose satisfactorily. Occasional attempts (13, 14<sup>1</sup>) to utilize the test when the basal concentration was elevated have yielded results which are difficult to interpret.

In extending the range of disorders to which the bilirubin excretion test can be applied, it is necessary to recognize that probably no test of excretory function can yield meaningful results when the plasma van den Bergh reaction is direct. The direct reaction is generally assumed to mean that bile is regurgitating from the biliary tract into the blood; if the assumption is true, a part of the material excreted by the liver is returned to the blood and estimations of blood level cannot be expected to measure excretion alone. In hemolytic disease the reaction is indirect, there is no evidence for regurgitation, and it should be possible to assess excretory function even though the basal concentration is high. The theory to be described provides a rational basis for interpreting the test in this type of case; the estimate of liver function which it yields is quantitative and independent of the basal concentration of bilirubin in the plasma.

## THEORY

Three assumptions are required in developing the theory of bilirubin clearance. Two of these

<sup>1</sup> These investigators studied the liver function of a patient with chronic hyperbilirubinemia, moderate splenomegaly and hepatomegaly, and march hemoglobinuria. The conventional bilirubin excretion test showed no retention of injected pigment after 3 hours, that is, plasma bilirubin concentration had returned to its previous equilibrium level of 3.0 mgm. per cent. Later, a bilirubin clearance test performed by the method of this paper disclosed marked impairment of hepatic excretory function.

TABLE II

*Findings with 18 patients in whom the velocity constant of bilirubin clearance,  $K$ , was less than  $5.0 \times 10^{-3}$  mgm. per minute per milligram-squared of concentration in the plasma*

Patient	Age	$L_{eq.}$	$K$	Diagnosis
	years	mgm. per 100 cc.	mgm. units $\times 10^{-3}$	
1. S.F.....	2	0.86	4.88	Bronchitis
2. D.S.....	1	0.50	4.69	Intracranial angioma
3. S.D.....	1	0.72	4.54	Behavior disorder
4. A.R.....	4	0.73	4.50	Epilepsy
5. A.C.....	6	0.52	3.90	Nephritis
6. F.B.....	7	0.73	3.63	Nephritis
7. R.H.....	11	0.36	3.36	Cirrhosis
8. R.F.....	11	0.99	3.35	Nephrosis
9. M.K.....	7	0.59	3.35	Diabetes
10. H.R.....	9	0.92	2.62	Intestinal parasites
11. H.S.....	12	0.76	2.36	Osteodystrophy
12. A.G.....	$\frac{2}{3}$	1.80	2.33	Erythroblastic anemia
13. R.C.....	2	0.69	1.92	Cirrhosis
14. B.S.....	9	0.86	1.45	Cirrhosis
15. W.B.....	13	1.84	1.39	Sickle cell anemia
16. L.H.....	9	0.55	1.38	Cirrhosis
17. R.R.....	6	2.45	1.23	Erythroblastic anemia
18. A.L.....	15	1.40	1.09	Hemolytic anemia (unclassified)

not greatly impaired. Patients 8, 9, and 10, with constants from  $3.35$  to  $2.62 \times 10^{-3}$  mgm. units, presented no other finding to suggest hepatic defect. Patient 11 exhibited the peculiar syndrome of asymmetrical osteodystrophy with areas of pigmented skin which has been described by McCune (15) and by Albright (16). She herself showed no sign definitely attributable to liver malfunction; however, other patients with the same syndrome (15, 17) have given a story of severe and protracted neonatal icterus. The last seven cases in the table represent instances either of chronic hemolytic anemia and hyperbilirubinemia or of cirrhosis. In all of these there is reason to think that excretory hepatic function was impaired.

Review of all of the findings indicates first that excretory liver function exhibits in different subjects variations through a fairly wide range before it can be designated as impaired. It follows that some patients may as the result of disease suffer considerable loss of this power and still retain more adequate function than other normal subjects. The range of excretory power in individuals with functionally adequate livers aids in an understanding of the wide range of serum bilirubin levels encountered in normal subjects. In our experience these levels have varied from  $0.21$  to  $0.99$  mgm. per  $100$  cc. The findings also suggest that values for the excretory constant below  $2.0 \times 10^{-3}$  mgm. units must be regarded as evidence of impaired function. With values between  $2.0$  and  $2.5 \times 10^{-3}$  mgm. units, it is probable that function has been damaged. With constants

above  $2.5 \times 10^{-3}$  mgm. units, the significance of individual findings is less clear. If all of the constants above  $2.5 \times 10^{-3}$  mgm. units, with the exception of the finding in the cirrhotic patient (R. H.), are accepted as apt to be encountered in the absence of hepatic defect, there are 26 records to determine the range and distribution of normal values. In mgm. units  $\times 10^{-3}$  these records disclose:  $2.5$  to  $4.0$ , 5 cases;  $4.0$  to  $5.5$ , 8 cases;  $5.5$  to  $7.0$ , 5 cases;  $7.0$  to  $8.5$ , 4 cases;  $8.5$  to  $10.0$ , 1 case; above  $10.0$ , 3 cases.

## SUMMARY

The rate of removal from the circulation of intravenously injected bilirubin can be evaluated as a "velocity constant of excretion" which provides a measure of the excretory function of the liver. The measure is not affected, as is the older type of bilirubin excretion test, by elevation of the basal plasma concentration.

Evaluation of the velocity constant is based on the assumption that normally circulating bilirubin depends on an equilibrium between the rate of formation, which is constant, and the rate of excretion, which has been observed to be approximately proportional at any moment to the square of the concentration in the plasma.

The velocity constant is computed in milligram units which express the rate of excretion from each  $100$  cc. of plasma in milligrams per minute per milligram-squared of concentration in the circulating plasma. A minimum of three determinations of serum bilirubin concentration is required for the calculation. The level before injecting bilirubin,  $L_{eq.}$ , is measured first. The injection,  $5$  mgm. per kilogram of body weight, is then given. After a lapse of  $5$  to  $7$  minutes for mixing in the circulation, a second sample,  $L_1$ , is obtained. Finally, when from  $2$  to  $4$  hours have elapsed, the third sample,  $L_2$ , is withdrawn. If  $t_1$  and  $t_2$  represent the time in minutes corresponding to the samples  $L_1$  and  $L_2$ , which express concentration in milligrams per  $100$  cc. serum, the value of the velocity constant,  $K$ , can be computed from the equation:

$$K = \frac{1.1513}{L_{eq.}(t_2 - t_1)} \log \frac{(L_2 + L_{eq.})(L_1 - L_{eq.})}{(L_2 - L_{eq.})(L_1 + L_{eq.})}.$$

A study of 35 patients with normal and defective livers revealed variations in the velocity constant from  $1.09$  to  $24.5 \times 10^{-3}$  mgm. units.

For the present, values above  $2.5 \times 10^{-3}$  mgm. units must be interpreted as evidence of normal hepatic function, although low constants within the normal range may for some patients be indicative of loss of excretory power. Values between  $2.0$  and  $2.5 \times 10^{-3}$  mgm. units provide presumptive evidence of damaged hepatic function and values below  $2.0 \times 10^{-3}$  mgm. units are positive evidence that function is impaired.

#### APPENDIX—METHOD OF ANALYSIS

The assessment of hepatic function by means of bilirubin clearance depends upon accuracy in determining bilirubin in serum or plasma. In common with other investigators (1, 3) we have found that the requisite precision is not obtainable through measurement of the color developed in the van den Bergh reaction. This is true even when the azobilirubin is estimated photoelectrically by the excellent method of Malloy and Evelyn (18). Accordingly, we have followed the suggestions of Ernst and Förster (19) and of Eilbott (1) in using acetone to precipitate serum protein so that the yellow color of bilirubin itself can be measured in the filtrate. The sensitivity of the method and the accuracy of the results have been increased significantly by use of a selective color filter and photoelectric analysis. Previous investigators who have used the method for measuring bilirubin excretion have not been concerned with the error due to the yellow color of lipochromes; such carotenoid pigments presumably do not vary throughout the test and their absolute magnitude was unimportant since only increments in bilirubin concentration above the basal level were used in computing percentage retention. Measurement of the velocity of bilirubin clearance, however, depends upon the absolute concentration of bilirubin at all times during the test. Accordingly, carotenoid pigments have been estimated separately after extracting the serum with petroleum ether; a correction is then applied in the measurement of bilirubin.

*Estimation of total yellow color (bilirubin plus lipochrome).* Two and five-tenths cc. of distilled water, 0.5 cc. of the serum, and 10 cc. of redistilled acetone are pipetted in the order mentioned into a 15 cc. centrifuge tube. The contents are mixed thoroughly with a stirring rod which is subsequently discarded so that the tube can be closed with a rubber cap. It is then placed in the icebox for 15 minutes or until well chilled. Throughout these and later manipulations every effort is made to minimize exposure to light. We have routinely stored samples and tubes in a covered can, removing them only in subdued light for essential steps in the procedure. Centrifugation of the chilled tubes is carried out for 15 minutes in 300 cc. bronze cups which are filled with cracked ice before the tubes are inserted. Immediately afterwards the supernatant fluid is transferred to another tube which is stoppered and allowed to come to room temperature. The purpose of chilling during this phase of the procedure is to obtain a solution free from any

trace of turbidity. When chilling is neglected the fluid will become warm while in the centrifuge and, even though clear when first removed, may later develop a slight cloudiness as the temperature falls. The final step involves the photoelectric reading.

*Estimation of the yellow of lipochrome.* Since it is assumed that the amount of carotenoid pigment in the serum does not vary during the test, the portions of the serial samples which remain after the estimation of total yellow color are mixed to provide a single sample on which lipochrome is measured. In principle the analysis follows the procedure of Stoner (20). Two cc. of the serum is transferred to a test tube; anhydrous calcium sulphate is added and by means of a glass spatula thoroughly mixed with the serum until a dry paste results. Two cc. of 95 per cent alcohol is stirred thoroughly with the paste. Then, exactly 8 cc. of petroleum ether is added; the tube is stoppered and the contents shaken vigorously for several minutes. Upon settling, a portion of the petroleum ether layer, which now contains the carotenoid pigments, is removed for measurement of color intensity in the photoelectric instrument.

With both analyses, total yellow color and lipochrome, the galvanometer readings are calculated to express in milligrams per 100 cc. of serum the quantity of bilirubin which would have yielded the same readings. Bilirubin is then computed as total yellow color minus the yellow due to lipochrome. With most subjects the correction for lipochrome is small, in the neighborhood of 0.1 or 0.2 mgm. per 100 cc. In one patient only in our series, a diabetic, the correction reached a value of 0.67 mgm. per 100 cc.

The photoelectric photometer used for the estimation of color intensity was one which was constructed in our own laboratory (21); other instruments which utilize selective color filters should be adaptable to the procedure. Readings were made in optical cells which were either 2.5 or 5.0 cm. in length; the shorter cell was used when color intensity was high and the longer cell with paler solutions. The filter, which furnishes blue light, was constructed of the following components: Corning Number 511, violet glass, thickness 3 mm.; Jena optical filter glass GG-5, thickness 2 mm.; Corning light shade Aklo glass Number 396, thickness 2 mm. Solutions of bilirubin exhibit maximum light absorption at  $440 m\mu$ ; the filter described transmits light from 410 to  $490 m\mu$ , with a peak transmission at  $450 m\mu$ .

Calibration presented a problem. When different standard solutions are prepared by dissolving weighed amounts of bilirubin in chloroform and subsequently diluting either with alcohol or acetone, discrepancies among replicate readings will be much greater than when multiple measurements are made on the same solution. This is especially so when the bilirubin of one manufacturer is compared with that of another. We have assumed, then, that part of the difficulty arises from impurities in available samples of bilirubin; another part undoubtedly comes from the circumstance that bilirubin in either alcohol or acetone is not in true solution but rather in a state of colloidal dispersion. Variation in the size of such colloidal aggregates is known to influence light absorption

(22). On the other hand, calibration curves prepared from dilutions of solutions showing the same initial color intensity have always agreed closely; likewise, the agreement between duplicate analyses of serum has been excellent. Evidently, then, the method is capable of yielding results whose relative precision is high; the absolute accuracy of the levels is not better than can be obtained by other good technics. These circumstances determined the method of calibration. The relative value of readings on the galvanometer scale was first determined carefully. The absolute concentration of the solutions of bilirubin used for this purpose was not known. Carefully prepared dilutions of each solution revealed slight but clearly demonstrable deviation from Beer's law. The deviations were calibrated so that color intensity could be measured in arbitrary units of bilirubin. Finally, the number of milligrams corresponding to each arbitrary unit was determined in the following way: Twenty-four samples of serum which contained varying amounts of indirect bilirubin were analyzed by the procedure outlined above and by the photoelectric method of Malloy and Evelyn (18). The latter method had previously been calibrated in our own laboratory with pure Hoffmann-La Roche bilirubin. The average findings by the two methods were then used to determine the milligrams to be assigned to each unit. An important circumstance in leading us to adopt this comparative method of calibration was the fact that Hoffmann-La Roche bilirubin can no longer be purchased on the market. Malloy and Evelyn have presented reasons for believing this bilirubin to be of a higher grade of purity than that procurable from other sources.

Practically all of our analyses of serum have been performed in duplicate. The relative accuracy of the present method is indicated by the agreement between the duplicates. With levels of bilirubin below 1.0 mgm. per 100 cc., the data reveal a standard error of analysis, S.E.A., of  $\pm 0.012$  mgm. per 100 cc. With higher concentrations, the errors per 100 cc. are as follows: 1.0 to 2.0 mgm., S.E.A. = 0.023 mgm.; 2.0 to 5.0 mgm., S.E.A. = 0.029 mgm.; above 5.0 mgm., S.E.A. = 0.045 mgm. The values yielded by the method do not appear to be affected by hemolysis in the sample. We have obtained identical results in serum analyzed before and after the addition of laked red blood cells. In contrast, such additions have always decreased the intensity of color in the van den Bergh reaction. The method cannot be used to measure bilirubin in the serum of patients with obstructive jaundice or with icterus due to hepatitis. A part of the bilirubin in these cases gives a direct reaction in the van den Bergh test; direct bilirubin is precipitated by acetone along with the serum proteins. Under such circumstances the protein precipitate is stained yellow and the fact that the estimate will be unreliable can be recognized at once.

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# MEASUREMENT OF THE SENSITIVITY OF THE SMALLEST BLOOD VESSELS IN HUMAN SKIN: RESPONSES TO GRADED MECHANICAL STIMULATION IN NORMAL MEN<sup>1</sup>

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The physiological responses of the smallest blood vessels in human skin to mechanical and chemical stimuli have been the subject of numerous studies and several important monographs (1, 2, 3, and others). The qualitative aspects of the white and red reactions of the triple response have been well-defined and their localization to the smallest vessels has been established. Despite the extent of, and widespread interest in this subject, no attempt has been made until now to put these on a simple quantitative basis. Such experiments should enable an observer to measure the sensitivity of the skin capillaries in a subject from day to day, and to compare such data among a group of individuals. The present report offers one of two such methods which we have developed.

The procedure usually employed experimentally to elicit the several components of the triple response are mechanical pressure and local infiltration (by pricking or iontophoresis) of vasodilator or vasoconstrictor drugs. The chemical methods have been used in a quasi-quantitative manner (4, 5, 6) but these tests depend upon a number of factors which may only indirectly involve the sensitivity of the capillaries. Plethysmographic studies and measurements of skin temperature likewise fail to reflect the irritability of the smallest vessels (7, 8). The response of these vessels to graded mechanical stimulation offers, on the contrary, a direct approach to this problem. The technic described below depends upon this principle. Its application rests upon control of two qualities of a stimulus, namely, intensity and duration. Thus, if a true measure of the excitability of the vessels were obtained, a typical strength-duration curve like that found in determining the threshold of

excitability in nerve or muscle would result. The apparatus described below yields such data simply and quickly.

## METHOD AND PROCEDURES

*Apparatus.* This consists of a machine which varies two factors in a critical manner. The intensity factor is varied manually by changing the weights on a skin stroker. The duration factor is varied by having a device which will move this loaded skin stroker over the forearm at a suitable range of speeds. The duration of the stimulus over any portion of the skin may then be determined by calculation from the rate. In practice, a range of stimulus-durations of 12 to 14 seconds per centimeter to 0.03 seconds per centimeter was found to be necessary. Different speeds of the piston were obtained by controlling the outflow of compressed air in front of a piston on which a constant pressure is exerted. By glancing at Figure 1, it will be seen that moving the carriage toward the reader would move the piston into the cylinder in the opposite direction, thus compressing the column of air in the cylinder. By adjusting the air-escape valve at a previously determined position, the piston can be made to move at a given rate down the cylinder provided the tension exerted by the rubber motor remains nearly constant.

The technical details are simple. An ordinary tire pump provides the piston and cylinder. A section nine inches in length is cut off, leaving a blind cylinder with an air outlet. The rod which is attached to the piston is cut off and a length of number 22 gauge piano wire is connected to both sides of the piston. The cable is led off on the compressing side through the end-plate of the cylinder (drilled so as to provide an air seal). Both ends of the cable are looped over ball bearing pulleys and attached to a small turnbuckle on the carriage. The escape valve is a vital part of the machine and must be mechanically perfect. It must permit fine gradations in air-escape, since slight changes in this respect cause marked variations in the speed of piston travel. A valve consisting of two flat discs of number 18 gauge copper one and one-half inches in diameter and ground perfectly flat on one side was found suitable. In one disc, a hole five-thirty-seconds of an inch in diameter is drilled one-half inch from the center; in the other, a slit three-eighths of an inch long is cut, placed one-half inch from the center, and tapered from one-eighth of an inch to a very fine point. When the ground surfaces of the discs are applied together it will be seen that as one disc is

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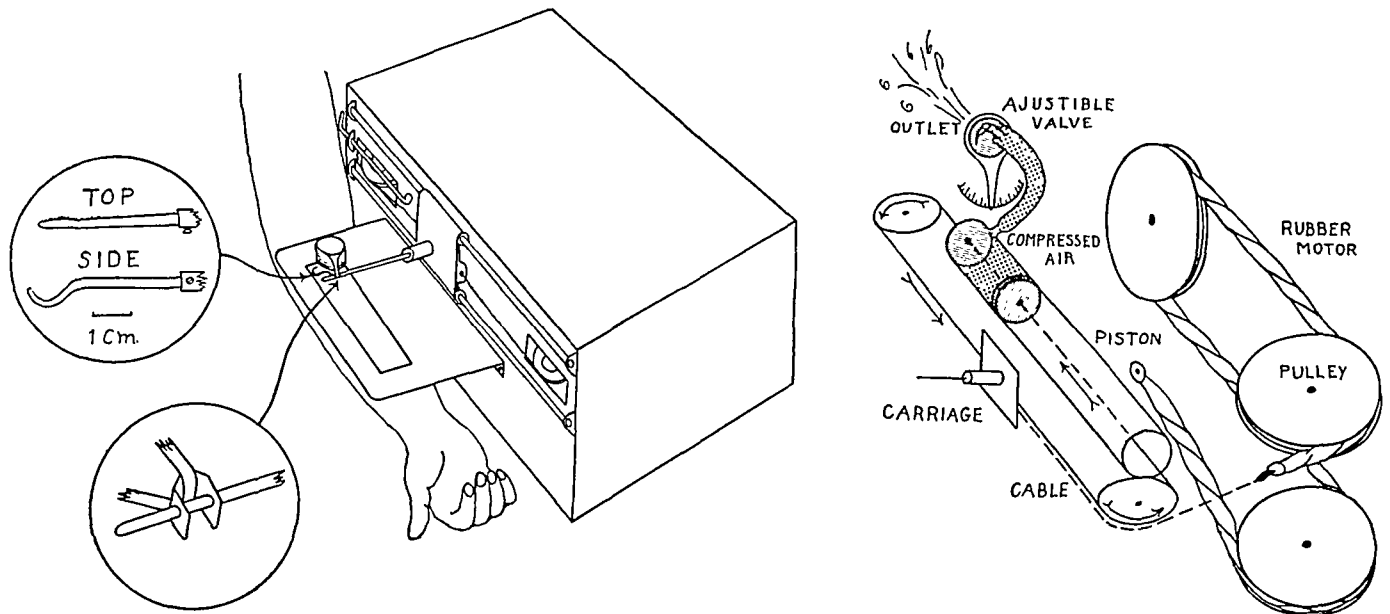


FIG. 1. DIAGRAM OF APPARATUS FOR APPLYING GRADED MECHANICAL STIMULATION TO HUMAN SKIN

Gradations in weight are obtained by employing selected weights in the carriage of the stroker; in speed, by adjusting the valve which controls the rate of escape of compressed air from the pump. See text for details.

rotated and the other held stationary, the tapering slit is gradually superimposed upon the circular orifice. An airtight connection is made between the circular orifice and the cylinder; a suitable handle is attached to the other disc, and a scale with one degree gradation is constructed. The discs are held together firmly by a spiral spring which runs over a central pivot. By means of brass tubing soldered to each end, the carriage of steel plate rides on tracks of two steel drill rods. A brass bushing holds the rod of the skin stroker. The carriage must run smoothly. An extra slip of cable is connected to the rubber motor, as illustrated. Rubber was used to provide a strong, even pull over a four-inch distance. When fifteen feet of one-fourth of an inch of flat rubber are looped over the four-inch pulleys in ten strands, as shown, this requirement is adequately filled. The skin stroker is a chromium plated brass rod five-thirty-seconds of an inch in diameter and shaped so as to provide a smooth stroking surface to the skin. The end is bent into an arc of 0.5 centimeter and tapered to a diameter of 2.5 millimeters. It should be carefully polished to eliminate all burrs. Such a stroker loaded with five hundred grams covers eighteen square millimeters ( $3 \times 6$ ) in area on the skin of the forearm. The loading pan is merely a wire basket, as shown in the diagram. In our apparatus it exerts a force of twenty-five grams. The whole apparatus is mounted in a box. A heavy sheet of metal with a rectangular cut-out four inches long and one and one-half inches wide is mounted below the stroker as an arm guard. It is covered underneath with velvet to avert cold reactions on the skin. The guard also serves to provide a surface on which the stroker runs before touching the skin; during this time, acceleration is completed at all speeds.

In calibrating the machine, a strip of thin metal ex-

actly one centimeter wide is attached to the carriage so that it stands upright. The machine is now interposed between a light source and an optical kymograph so that when the carriage moves the thin strip cuts a light beam for an interval of time dependent on its speed of travel. An ordinary electrocardiograph timer of 0.04 second per centimeter is also inserted on the record. The record thus obtained will show a black line with various segments cut out to represent different rates of speed. These rates may be calculated as duration of stimulus per centimeter and conveniently transposed to the scale on the pointer.

*Procedure in an experiment.* The subject is seated comfortably with a bared arm which is supported on a rubber wedge in place under the platform of the apparatus, as shown in Figure 1. In practice, it is found convenient to have the subject in such a position that the stroker runs up the forearm instead of down, as shown in the diagram. The operator is then seated in position to adjust the valve weights and to reset the stroker. The observations are made under bright lights (two 100-watt bulbs, one blue and one yellow) in reflectors placed at a distance of about thirty inches from the apparatus. They are focused somewhat to one side in order that the full heat from the bulbs does not fall on the arm. The effect of local changes in temperature on the responses under observation will be discussed below. As a matter of record, notation is made of the skin temperature (number 30 gauge wire thermocouple and an L & N skin potentiometer), dry bulb, wet bulb, humidity, and barometric pressure.

For a determination, the stroker is released to move at any desired speed along a line two to four inches in length. The arm is then held toward the light and the changes in skin color are observed along the line of the stroke. These may vary in the following ways: With

relatively little weight, an area of pallor (constriction of the skin capillaries (2)), may be seen to develop in the course of 35 to 40 seconds. This fades away in some 2 to 5 minutes. When the pressure is heavier, a bright red line (capillary vasodilatation (2)), of even color and with sharp edges, surrounded by a broad pale area of vasoconstriction with sharply defined edges, is seen along the line of the stroke. Beyond this an irregularly defined flare may be present. Between these two responses is a third one which we have found convenient and easy to employ as the threshold response in measuring the sensitivity of the vascular reactions; it consists of the area of pallor seen in the two responses above plus a faint, mottled or incomplete red line along the line of the stroke. In judging this response, it is essential that a red tint, which acquires a color characteristic of oxygenated blood, be distinguished along the line, and that mere indentation of the skin is not mistaken for the threshold response. Little practice is necessary for this. Examples of the three responses are shown in Figure 2. They have been taken from a model of the forearm on which the tache reactions have been depicted by an artist.

For each successive stimulus-duration the threshold is re-determined, largely by trial and error, using various weights. A little experience shortens the time required for these determinations. After each trial, a new area of the arm is placed under the stroker plate and a reading made. Extensive trials have shown that the inner aspect of either forearm may be used interchangeably in making the points of the strength-duration curves. It is not necessary to clip or shave the hair from the arm; in fact, it is believed that this makes some skins less susceptible to the stimulus of the stroking.

## RESULTS

*The strength-duration curve.* An example of one of the strength-duration curves, determined in the above manner, is shown in Figure 3. In this, each point, indicated by a dot, is the threshold response. The shaded area indicates, at the top, the curve of appearance of the full maximal response (solid red line); at the bottom, the curve of maximal capillary vasoconstriction without the red line.<sup>2</sup>

The position of this curve on the same subject throughout the month of September, 1940, is shown in Figure 4. The similar contours and the fair agreement of the several curves may be seen by noting the relative positions of the various symbols for the separate curves. These curves, it may be mentioned, apparently were not affected in their relation to each other by room temperature (wet or dry bulb), skin temperature, humidity or barometric pressure.

Thus far, curves similar to these (sixteen of which are shown in Figure 5) have been obtained

<sup>2</sup> The third point on the curve followed immediately after a period of increased activity, as the subject was called from the room. By the time the next point (second from the left) was determined, recovery had occurred.



FIG. 2. PHOTOGRAPH, MADE FROM MODEL OF AN ARM, SHOWING THE THRESHOLD RESPONSE, SUB-THRESHOLD RESPONSE, AND SUPER-THRESHOLD RESPONSE

The threshold response is represented by a broken, uneven red line of vasodilatation surrounded by a definite but limited pale area of vasoconstriction, the sub-threshold response by a pale area of vasoconstriction only, and the super-threshold response by a strong, continuous red line of vasodilatation, surrounded by a strong, wide area of vasoconstriction; beyond this, as shown here, there may be a red, reflex arteriolar flare.



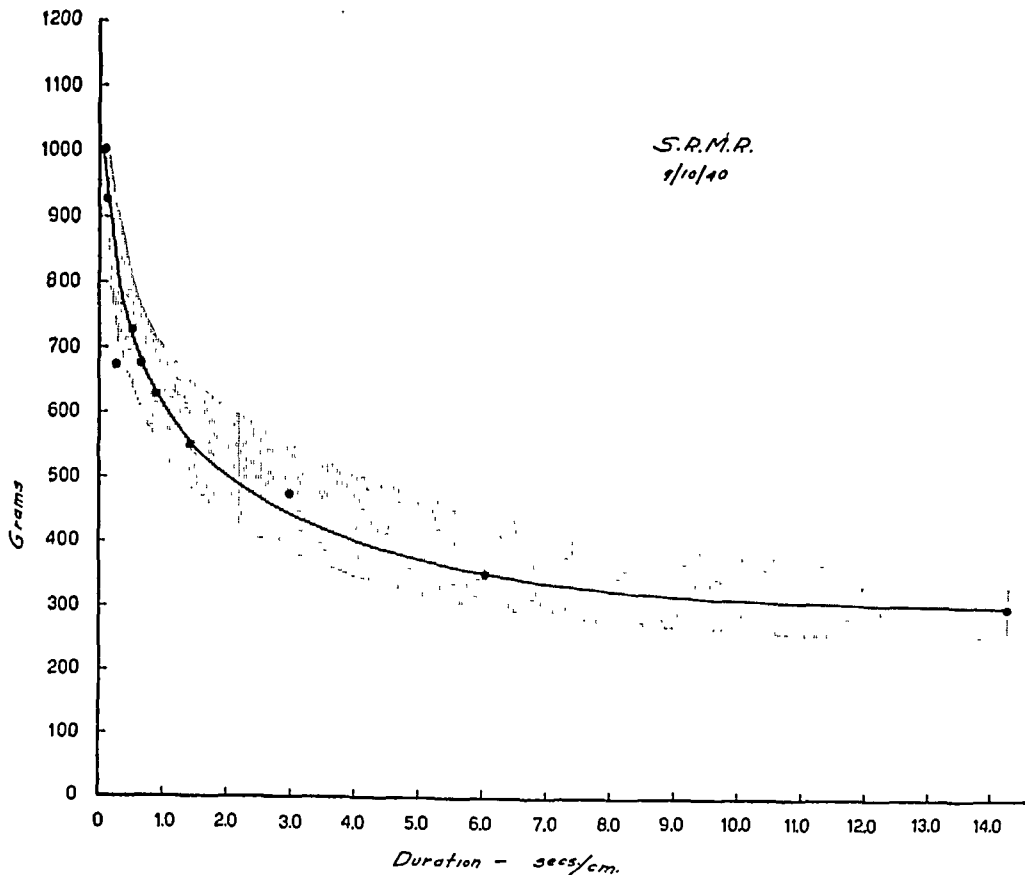


FIG. 3. A CHARACTERISTIC TIME-INTENSITY CURVE OF THE THRESHOLD RESPONSE

The dots indicate actual determinations of the response. The shaded area signifies, at the top, the level at which the super-threshold response is obtained; below, the level at which the sub-threshold (constriction only) is strongest, without a trace of the red. Vasoconstriction may actually be detected faintly down to 20 to 50 grams at any of these speeds. The third point from the left was made immediately after the subject returned to the room, after being called away.

on thirty-eight normal men.<sup>3</sup> Where more than one curve was obtained, each represents the aver-

<sup>3</sup> The choice of males was made by random sampling, and medical students from the first to the fourth years were selected as subjects. None was known to be abnormal or unhealthy in any way. In addition, members of the teaching and technical staff served as subjects. Thirty-five were in the third decade of life and three in the fourth decade. Additional data were obtained on half as many more subjects, but these are not included in Table I since the curves are incomplete. (The data were obtained at a time when it was believed that another method of treatment, which utilized only the region of the inflection of the curve, was possible.) Insofar as they go, these data compare with the more complete curves reported here. It was impossible to obtain curves on only two subjects in the entire group. One of these, a red-headed subject who has never tanned, gave atypical responses throughout the range of stimulus-durations. These were characterized by unusually intense and large areas of erythema along the line of the stroke, without the surrounding area of pallor. The second atypical sub-

age of all the curves for any individual. Attention may be called to several features of this group of curves. First, the point of inflection on most curves was obtained at a stimulus-duration of 1 to 2 seconds per centimeter, ten of these requiring between three to four hundred grams to induce the threshold response. The remaining six curves, each of which was typical for the individual from whom it was obtained, showed somewhat different thresholds at the point of inflection. To what this may be attributed, we are unable to say at the present time. Second, with the exception of two curves, the weights necessary for threshold responses at the fastest speed was between five hundred and twenty and eight hundred grams. In the

subject was periodically catatonic. His responses did not appear for from 2 to 5 minutes and they persisted for about half an hour. This is in contrast to the threshold responses in normal subjects which are usually maximal within 40 to 60 seconds and subside within several minutes.

other two subjects, it was one kilogram. Finally, considerable spread was observed in the weights necessary for threshold responses at the slower speeds. Perhaps this may be ascribed partly to variations in skin thickness or water-content which the heavier weights would circumvent at higher speeds. But its true cause is not known.

*Proof of the threshold nature of the strength-duration curve.* The response which we have adopted as "threshold" is complex, consisting of an area of vasodilatation against a background of vasoconstriction. Doubt naturally arises, therefore, concerning the significance of the higher stimulus-intensities (weights) required at the faster speeds. May not the increased weight be required to elicit more injury in order to produce, in turn, more vasodilating substance to overcome a stronger vasoconstriction? Alternatively, is the capillary constriction of equal intensity throughout the range of the strength-duration curve, and, hence, is the red line of vasodilatation the result of

an equal intensity of chemical inhibition throughout? The question is answered in favor of the latter possibility by the following experiments.

The thresholds were determined at four different speeds. They were then repeated at suitable intervals upon an arm in which various degrees of partial venous occlusion were maintained by a sphygmomanometer cuff applied immediately *before* laying down the stroke. Two such levels of occlusion were determined, one of which just served to prevent the halo of vasoconstriction along the line of the stroke, the other to permit, in diminished intensity and form, the halo of vasoconstriction. The purpose of this procedure was to show that, if the constriction were more forceful at the higher stimulus-intensities, then higher venous occlusion pressure would be necessary to prevent vasoconstriction than at the lower stimulus-intensities.

The result of one such experiment is shown in Figure 6. It will be seen that, regardless of the

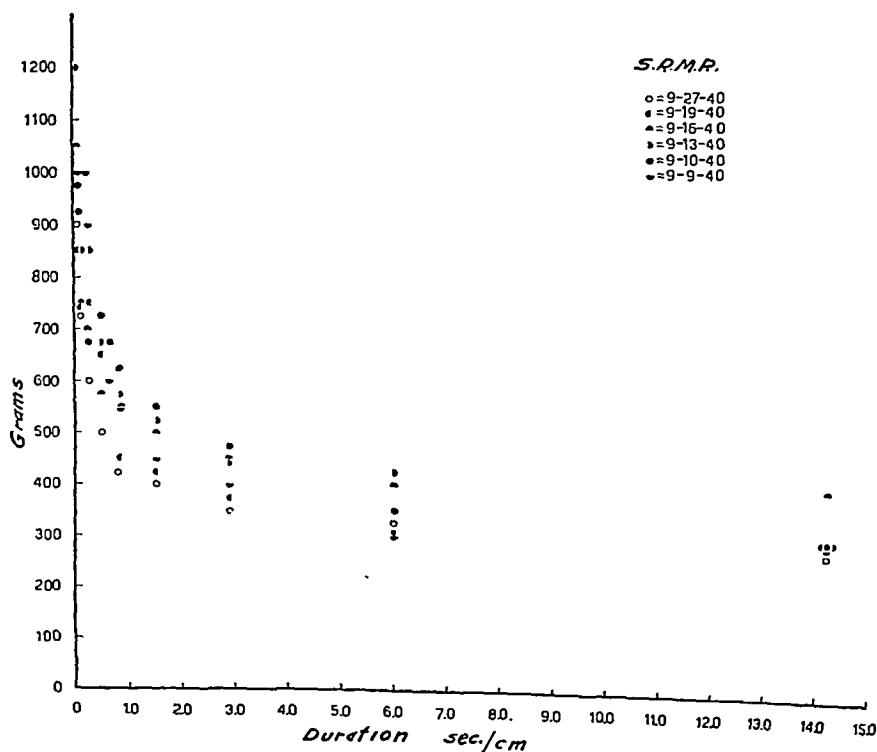


FIG. 4. TIME-INTENSITY CURVES OBTAINED FROM ONE SUBJECT DURING THE MONTH OF SEPTEMBER, 1940

There is variation, but this is in the intensity parameter of the several curves, as may be seen by noting the position of any curve by means of the symbols.

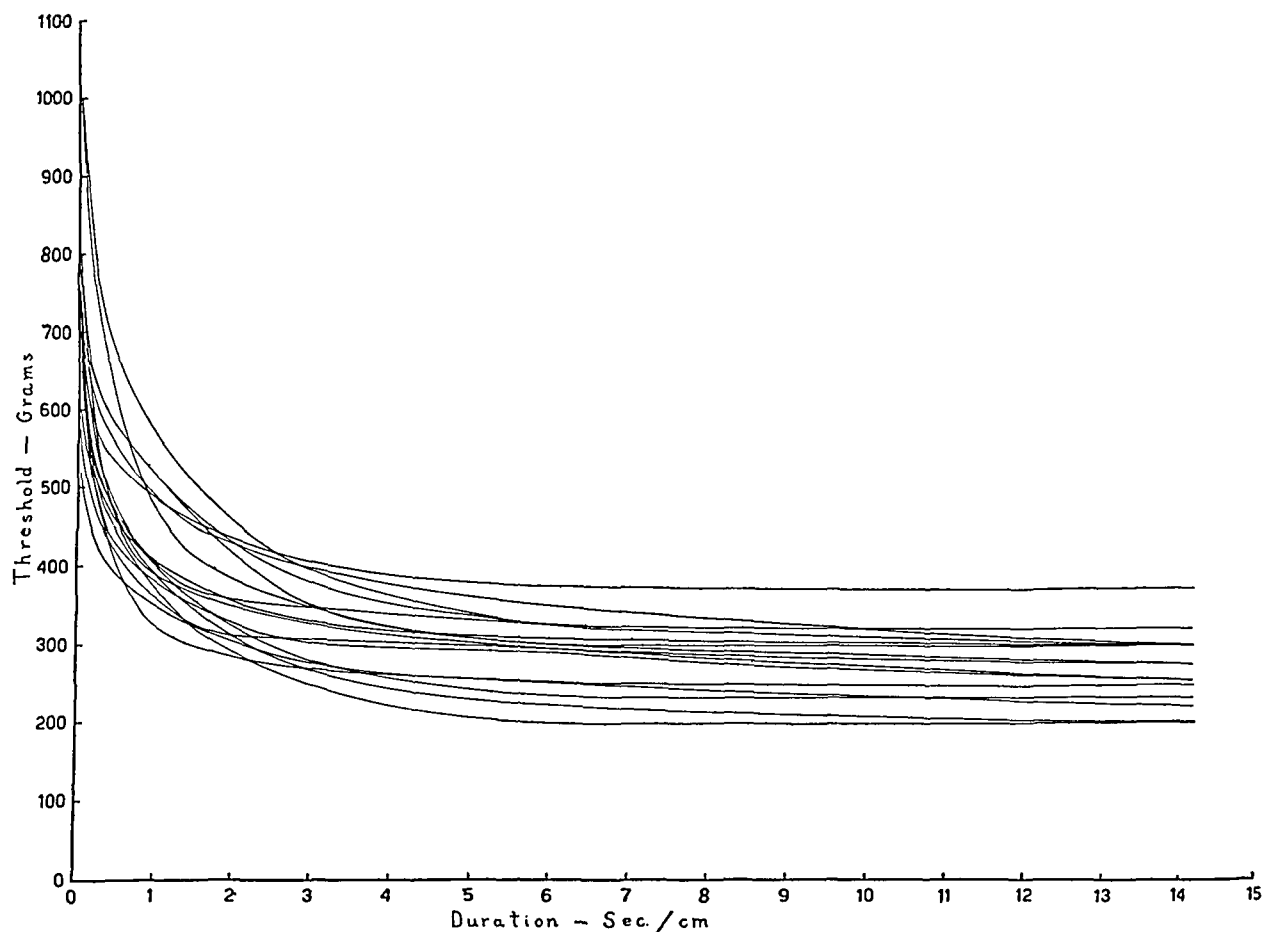


FIG. 5. SINGLE TIME-INTENSITY CURVES OBTAINED ON SIXTEEN DIFFERENT, PRESUMABLY NORMAL, HUMAN MALES

weights used, fifty millimeters of mercury prevented constriction, whereas forty millimeters of mercury permitted the development of discernible vasoconstriction. In either case, release of the venous circulation soon afterwards regularly permitted development of the response to nearly full intensity, thus showing that fatigue resulting from inadequate circulation had not occurred. Consequently, the conclusion follows that throughout the strength-duration curve described above, we are dealing with responses of about uniform intensity, and the curve is characteristic of the threshold for excitation of the capillary blood vessels to graded mechanical stimulation applied at appropriate rates.

Mention should be made of the fact that we have confirmed the observation of Lewis that, if the stimulus be strong enough, the white and red reactions will develop, even though the venous occlusion pressure approaches a diastolic level.

*Effect of complete venous stasis.* In view of the fact that responses may be obtained by ade-

quate stimulation with partial or complete venous occlusion, it was deemed advisable to investigate quantitatively the effect of stasis on the threshold of response. With complete stasis the circulatory conditions differ markedly from those in partial occlusion. In the latter, the blood flow is momentarily impeded and then continues at a higher pressure level (2), whereas in the former, the capillary blood pressure is materially lowered when arterial inflow is rapidly cut off by pressure.

The effect of circulatory stasis upon the excitability of the smallest vessels in the skin is shown in Figure 7. In this, as in other similar experiments, threshold stimuli were determined at three different speeds following periods of stasis lasting from 1 to 6 minutes. It is clear that circulatory arrest for these periods of time does not abolish the contractility of the smallest blood vessels (confirming Lewis) but it does raise appreciably and progressively their threshold for excitation. The effect is more pronounced when the stimulus is applied quickly.

To determine why circulatory arrest raises the threshold of the white and red reactions, a further investigation was necessary in order to distinguish between the effects of anoxia and of the action of metabolites and vasodilating substances which are known to accumulate in tissues in which the circulation is arrested.

*Effect of anoxemia on the threshold.* In this experiment, the subject rebreathed into and out of a closed tank filled with room air. The threshold of the skin response was measured, for one speed only, at intervals during the experiment. Expired air passed through soda lime to remove carbon dioxide. By means of suitable procedures, the oxygen in the expired air was estimated from time to time, and records of systolic pressure, diastolic pressure, heart rate, respiratory rate, and tidal air were made. From the latter, the minute volume of air respired was estimated.

The effects of diminishing oxygen tension in the inspired air are shown in Figure 8a. It will

be seen that within 10 minutes, when the oxygen was reduced to about 17 to 18 per cent, a perceptible elevation of the threshold of blood vessel reactivity occurred. There was little effect on blood pressure, heart rate or respiratory rate and depth at this time. As the oxygen in inspired air reached a level of 13 to 14 per cent, however, changes in all the above were recorded, particularly with regard to heart rate and respiratory rate. At about this time, a very abrupt increase in the threshold of the blood vessel reactivity occurred. Anoxemia without stasis, therefore, lowers the sensitivity of the smallest blood vessels in the skin and this is observed before important systemic circulatory and respiratory changes take place.

*Effect of hypercapnia on the threshold.* The effect of increased carbon dioxide in the expired air presented an interesting contrast to the effect of anoxemia. In Figure 8b, it will be seen that a marked decrease occurred in the threshold of reactivity of the capillary blood vessels and, as in

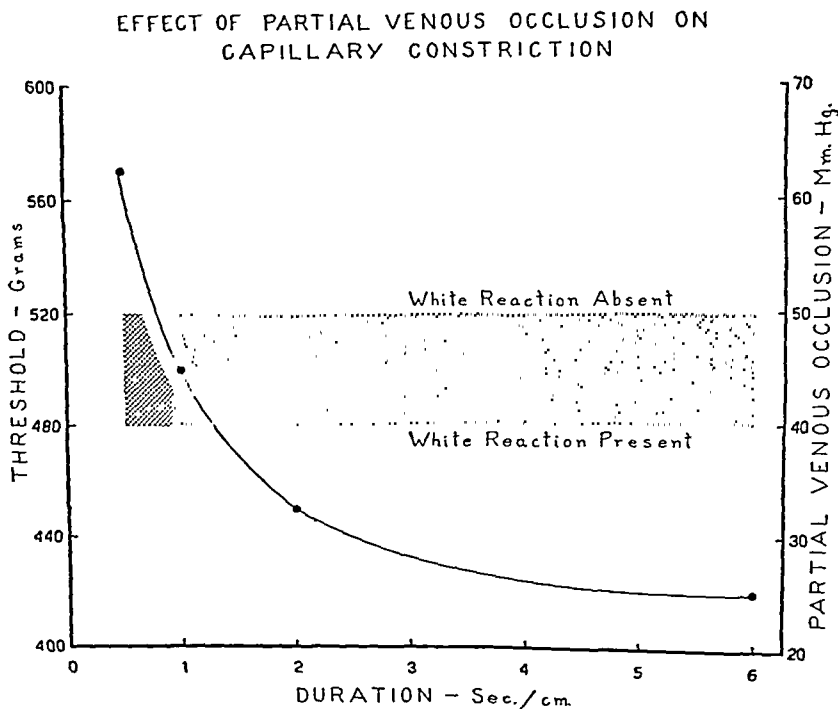


FIG. 6. PROOF THAT THE TIME-INTENSITY CURVES OF BLOOD VESSEL RESPONSE TO GRADED MECHANICAL STIMULATION ARE OF UNIFORM, THRESHOLD INTENSITY, AND DO NOT VARY WITH THE WEIGHTS, OR SPEEDS USED

Irrespective of the point on the curve, 50 mm. partial venous occlusion pressure, applied just prior to laying down of the stroke, prevented the zone of vasoconstriction, whereas 40 mm. permitted it.

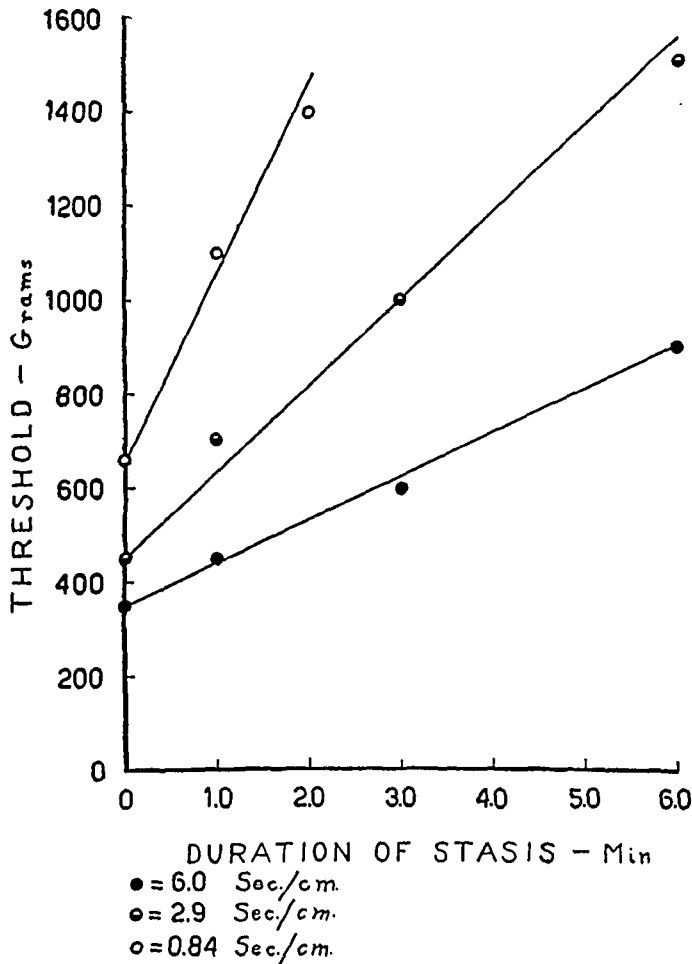


FIG. 7. EFFECT OF COMPLETE CIRCULATORY STASIS ON THE THRESHOLD RESPONSE

At each of three different speeds, the weight necessary for the threshold response increased with increasing duration of stasis.

the anoxemia experiment, this change in threshold was well-marked before important changes in heart rate, blood pressure or respiratory activity took place. Accordingly, these results attest a remarkable sensitivity of the capillary blood vessels to small changes in the gas-content of the blood.

*Effect of local temperature changes on the threshold.* The effect of increase in the local skin temperature on the end-point of the reaction (*i.e.*, the *equilibrium* point, not the *rate*) was studied to see if it would be necessary to control rigidly the room temperature during a series of determinations. The result is shown in Figure 9. The tissues were warmed gradually and held at any desired temperature for several minutes. When this was uniform (within 0.3 degrees Fahrenheit) along the line the stroker would take, the threshold was determined. It will be seen from the figure that the change was not very great, even though

the range of temperatures extended from 90 to 104 degrees Fahrenheit. This involved, in fact, a change in threshold of about 2 per cent per degree Fahrenheit. Inasmuch as the threshold determination is only read to within about  $\pm 20$  grams (*i.e.*, about 5 to 10 per cent) it may readily be seen that minor changes in room temperature will not seriously affect the shape or position of the strength-duration curve. New experiments, taking into account the *rate* at which the threshold equilibrium is attained at each temperature, will be required in order to establish, by the Arrhenius (9) equation, the chemical or physical nature of the effect of temperature on the reactivity of the blood vessels to mechanical stimulation.

#### DISCUSSION

*Criteria for evaluating excitability of the smallest blood vessels.* The results summarized above raise an important question, namely, in what manner may the individual strength-duration curves be employed to indicate relative degrees of blood-vessel excitability in a given person from day to day, or between different subjects? Examination of the curves in Figure 5 demonstrates the impracticability of using a simple measure like a chronaxie value, inasmuch as some curves with a high intensity parameter have a very short-time parameter and vice versa, while some curves cross others at one or two points. Davis and Forbes (10) have reviewed the limitations of chronaxie values. By replotting the data, however, it is evident that each of these curves is characteristic of the strength-duration curves which are obtained in the responses of other types of tissue (*e.g.*, smooth and striated muscle, nerve) to electrical stimulation (10, 11). This is demonstrated by plotting the data of the strength-duration curve on double logarithm paper. When this is done, as in Figure 10, it is found that each curve falls along a straight line. This occurs when the duration of the stimulus is between 0.25 to 2, 3 or more seconds per centimeter. This was true for stimulus-durations of about one second per centimeter in two curves only. The slopes of these and other similar curves (Table I) range from 0.19 to 0.55, with an average of 0.35. This is less than the range of 0.5 to 1.0 second per centimeter which is found for a variety of tissues in response to electrical stimulation (10).

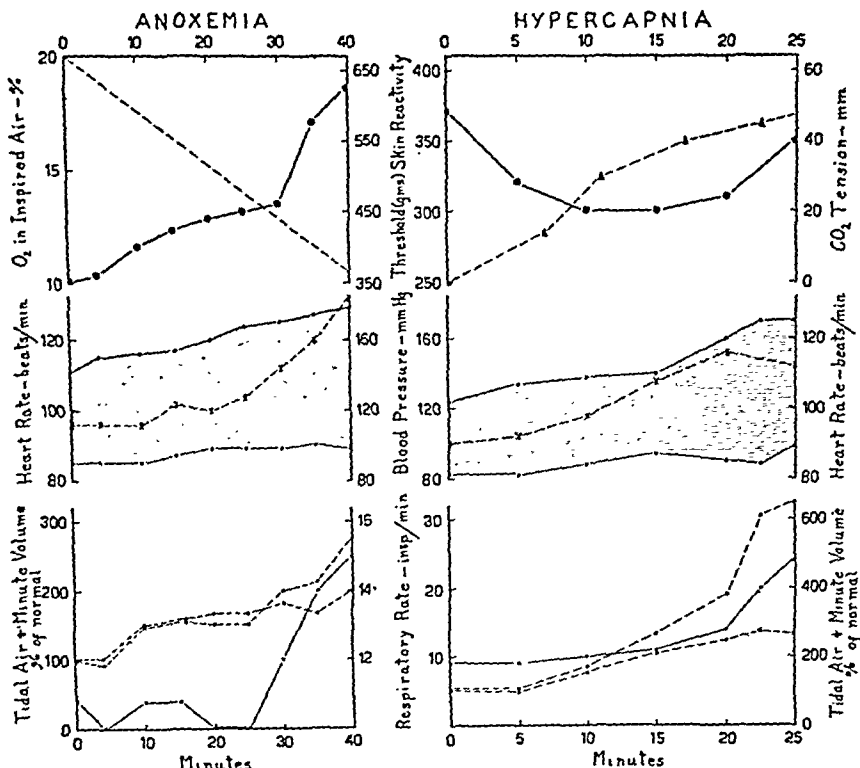


FIG. 8. THE EFFECT OF ANOXEMIA (A) AND OF HYPERCAPNIA (B) ON THE THRESHOLD RESPONSE (CONSTANT SPEED, VARIABLE WEIGHTS)

Heavy dots, threshold; crosses, heart rate; stippled areas, pulse pressure lying between systolic and diastolic pressures. Bottom, continuous line, respiratory rate; circles, minute volume index (%); dots, tidal air (%). These data show that changes in the threshold of blood vessel reactivity occur, increasing in anoxemia, decreasing with hypercapnia; in each, the change is detectable before important changes in circulatory or respiratory activities occur.

The selection of an adequate "index of excitability" raises many imposing problems, and no entirely suitable or universally recognized method is available. Davis and Forbes (10) point out that chronaxie is an expression of excitability in terms of the time factor alone, and that this is entirely empirical. They suggest that the "index of excitability" of Lassalle (12) is more adequate since it embraces the intensity factor as well. Therefore, it involves measurement of excitability in terms of energy, and this would seem to offer a better basis for comparison of the relative excitabilities of different strength-duration curves for a single tissue when these are obtained under identical conditions (10). The Lassalle factor is expressed as the index of excitability which is proportional to the reciprocal of the product of the

rheobase squared and chronaxie

$$(i.e., E = \frac{1}{\text{rheobase}^2 \times \text{chronaxie}}).$$

This coefficient is calculated and given in Table IA for each of the curves thus far obtained to date on the skins of thirty-eight normal human males.<sup>3</sup> It will be seen that the most excitable blood vessels ( $1.6 \times 10^{-4}$ ) are twenty times as sensitive as the least excitable blood vessels ( $0.08 \times 10^{-4}$ ) and that the mean is  $0.53 \pm 0.49$ . The standard error of the mean is  $\pm 0.079$ . Comparable data for two or more curves on a single individual at intervals of a week to several months are given in Table IB. Here, too, it will be seen that some variation in excitability is found, although the range for any subject is far less than that for the group as a whole.

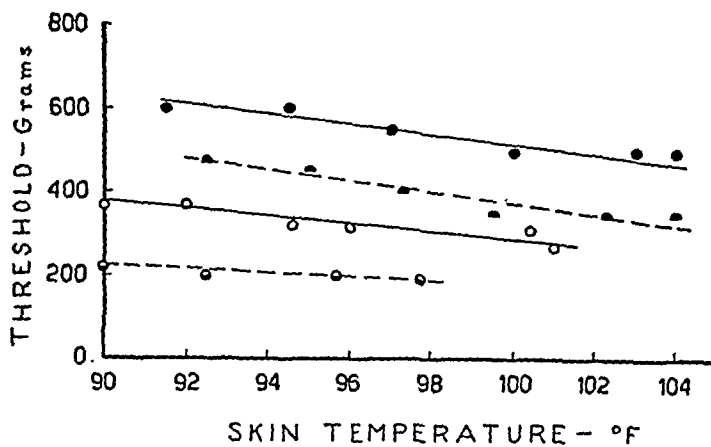


FIG. 9. EFFECT OF INCREASING SURFACE TEMPERATURE ALONG THE LINE OF STROKE ON THE THRESHOLD RESPONSE

Each of these indicates a shift in threshold of about 2 per cent per degree F. Since the response is read, usually, to about  $\pm 10$  per cent, small changes in temperature are relatively unimportant.

In view of the physiological factors which may modify the threshold of excitability in the blood vessels (a few of which are noted in this paper) it is not strange that this variation is found in single individuals and between different individuals.

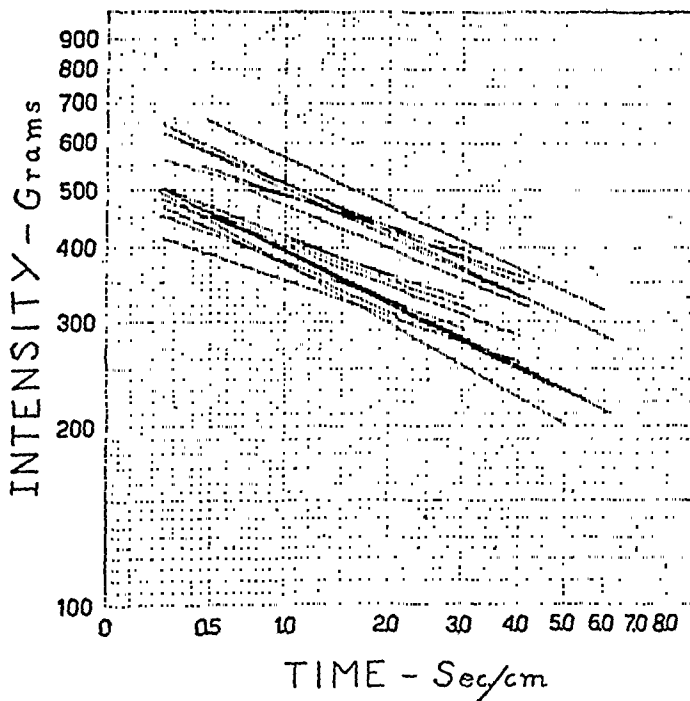


FIG. 10. THE TIME-INTENSITY CURVES OF FIGURE 5 PLOTTED ON DOUBLE COORDINATE LOGARITHM PAPER

The line is straight. At the right of each of the curves shown here the change in slope to the rheobase (omitted here for clarity) commences. Such curves are typical of the curves of electrical excitation of a number of tissues. The average slope here (see Table I) is 0.35.

TABLE I

Summary of the characteristics of the curves of threshold skin vascular reactions to graded mechanical stimulation in thirty-eight normal male subjects  
The coefficient of excitability is calculated according to the formula of Lassalle (12).

Slope log-log coordinates	Rheobase	Chronaxie	Coefficient of excitability: $E = \left( \frac{1}{\text{rheobase}^2 \times \text{chronaxie}} \right)$
A. DATA OBTAINED FROM DIFFERENT SUBJECTS			
	grams	seconds per centimeter	
0.45	180	3.90	0.08 ( $\times 10^{-4}$ )
0.26	260	1.50	0.10
0.50	150	4.50	0.10
0.31	220	1.85	0.11
0.40	160	3.50	0.11
0.37	250	1.00	0.16
0.42	350	0.50	0.16
0.50	280	0.70	0.18
0.55	280	0.60	0.19
0.44	170	1.80	0.19
0.45	275	0.67	0.19
0.50	325	0.49	0.19
0.29	310	0.50	0.21
0.28	200	1.00	0.25
0.33	170	1.30	0.26
0.38	200	0.85	0.29
0.25	220	0.70	0.29
0.40	300	0.37	0.30
0.50	300	0.35	0.31
0.47	200	0.75	0.33
0.38	250	0.37	0.43
0.51	300	0.25	0.44
0.28	250	0.35	0.46
0.38	170	0.60	0.58
0.43	250	0.25	0.64
0.45	300	0.15	0.74
0.23	275	0.18	0.74
0.38	250	0.20	0.80
0.35	350	0.10	0.82
0.30	275	0.15	0.88
0.23	325	0.10	0.94
0.19	320	0.10	0.97
0.40	320	0.10	0.98
0.28	300	0.10	1.11
0.23	275	0.10	1.33
0.50	375	0.05	1.42
0.28	250	0.10	1.60
0.33	250	0.10	1.60
Mean	0.35	260.1	0.78
S.D.	$\pm 0.098$	$\pm 57.5$	$\pm 1.01$
S.E. mean	$\pm 0.016$	$\pm 1.5$	$\pm 0.128$
t	21.9	173.3	6.09
P	$< 0.000000001$	$< 0.000000001$	$< 0.000000001$

B. CHARACTERISTICS OF TWO OR MORE CURVES OBTAINED IN FIVE SUBJECTS

Subject 1.			
September 9, 1940	300	0.60	0.18
September 10, 1940	300	1.00	0.11
September 16, 1940	400	0.08	0.78
September 19, 1940	300	0.50	0.22
September 27, 1940	270	0.35	0.39
January 2, 1941	200	2.00	0.13
January 20, 1941	220	1.90	0.19
January 21, 1941	250	1.40	0.11
Subject 2.			
September 27, 1940	200	1.40	0.18
October 4, 1940	200	0.85	0.29
October 11, 1940	275	0.10	1.32
October 23, 1940	250	0.25	0.64
Subject 3.			
September 26, 1940	250	0.25	0.64
October 1, 1940	225	0.60	0.33
Subject 4.			
September 13, 1940	300	0.20	0.55
October 7, 1940	250	0.10	1.60
Subject 5.			
September 20, 1940	325	0.10	0.94
October 24, 1940	225	0.15	1.32

The meaning of the data shown in Table I is indicated in the statistical treatment shown there. In the bottom line (Table IA), the factors P, calculated according to the recommendation of Mainland (13), has a value of less than 0.000,000,01. This signifies that, when observations from the same universe of data are obtained by random sampling in a group of similar subjects, a significant difference from the mean here given would occur only once in many thousands of samples. This mean, therefore, should serve as a base-line against which differences in reactivity of the skin vessels attributable to age, sex, disease or other condition may be shown with a high degree of confidence by adequate treatment of the data (13).

In conclusion, mention should be made of the fact that, in the plotting of every curve in Figure 10, a point is reached (the rheobase) at which the line curves and is parallel to the X axis. This is usual in curves of the strength-duration type (10, 11).

#### SUMMARY

A method for measuring the excitability of the smallest blood vessels in human skin is described. Two qualities of the stimulus, intensity (in grams) and duration (in seconds per centimeter), are varied at will. The threshold response consists of the white reaction of the triple response, in the middle of which is just discernible the beginning of the red reaction of the triple response. A typical strength-duration curve is found for each skin, and proof is offered to show that it represents liminal, or threshold responses throughout.

The effect of certain physiological variables upon the threshold response is established. The threshold is raised progressively with circulatory stasis; in systemic anoxemia, the threshold is raised, and in systemic hypercapnea it is lowered. In both cases these changes are well-marked before there is significant alteration in heart rate, systolic or diastolic blood pressure, or in respiratory rate, depth or minute volume. A change in

the temperature of the skin, by direct heating, brings about a change in threshold of about 2 per cent per degree Fahrenheit; the threshold responses are read to only 5 or 10 per cent, so that minor changes in room temperature during the course of an experiment do not affect the results seriously.

A procedure is described and applied to a series of data by which a coefficient of excitability is obtained. For the group of normal subjects, this offers a base line for comparison of the effects of experiment, disease or therapeutic procedure upon the sensitivity of the smallest vessels of the skin.

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# BLOOD IODINE IN PATIENTS WITH THYROID DISEASE<sup>1</sup>

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A reinvestigation of blood iodine in patients with thyroid disorders has been undertaken because it has been found that many of the older methods yielded erroneously high normal values. In a previous communication on methods for the determination of blood iodine (1), the authors reported normal values for 10 males and 10 females which ranged between 2.5 and 3.7 gamma per 100 cc., amounts which are lower and less variable than those reported by earlier investigators. They also noted in this paper that 4 groups of workers (2, 3, 4, 5) who had employed the recently revised acid ashing methods obtained similarly low values. Therefore, on the basis of the published criticisms (1, 2, 6, 7) of the alkaline ashing and the Leipter methods, and an investigation of the possible sources of error in the permanganate ashing method, the authors concluded that credence must be given to the low values for normal blood iodine.

In the present investigation the blood iodine levels have been determined in 55 patients with a number of different symptoms and signs of thyroid disorder. The results have been analyzed in an attempt to determine the relation of blood iodine to the symptoms and signs of hyperthyroidism.

## METHODS

The basal metabolic rates were determined with the Benedict-Roth apparatus under the standard conditions which have been described by Benedict, Dubois and others. In many instances, the basal metabolic rates obtained on the morning after the patient's admission to the hospital were omitted because it was feared that the patient was apprehensive or excited. In such instances, the values represent the determinations 2 to 4 days after admission to the hospital, when the patient was more adjusted to the basal metabolic test. In a few instances, Lugol's solution had been given for 3 days before these second metabolism tests were determined.

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Venous blood was taken under post-absorptive conditions and the serum analyzed for cholesterol by methods previously described (8, 9). Blood iodine was determined by the permanganate acid ashing method, which yields average recoveries of 95 per cent (1). No patient had had Lugol's solution, iodized salt or any form of iodine therapy during the 3 weeks before measurement of the blood iodine. Furthermore, no patient had had any diagnostic test in which an iodine-containing contrast medium was used, except B5469 (Table III) who had had lipiodol 10 years previously.

## DATA

The largest group (Table I) consists of 31 patients with definite hyperthyroidism. All the patients except the 3 at the end had thyroidectomies. The first 28 cases are listed in the numerical order of the hospital records. Tables II and III present data on patients with complications or nervous system disorders, in whom hyperthyroidism was considered or was doubtful. For comparison, the iodine has been determined in the blood of 6 patients (Table IV) with nodular enlargement of the gland but with no systemic signs of thyroid overactivity. The blood iodine has also been evaluated in 7 patients with signs and symptoms of myxedema.

Owing to the fact that clinical evaluation of the degree of thyroid overactivity is difficult and somewhat subjective, the diagnostic estimates were made by 2 or 3 physicians without knowledge of the blood iodine or serum lipid values. Subsequently, the 48 patients were divided into 4 groups in accordance with the presence or absence of thyroid enlargement, exophthalmos, tremor, restlessness, cardiac enlargement, tachycardia, warm, flushed skin, increased perspiration and malnutrition. In addition to these clinical symptoms, the height of the basal metabolic rate was an important criterion. The patient's response to iodine or thyroidectomy was also used in evaluating the original diagnosis.

The blood iodines, serum cholesterol and symptoms of hyperthyroidism of the patients at the time of admission to the hospital are given in the tables. Cases that are marked with asterisks presented certain unusual clinical features which are mentioned in the protocols.

Improvement of the patients is indicated in the 3 columns at the right of the tables. Basal metabolic rates after Lugol's, but before thyroidectomy, and after operation are given. Clinical improvement after Lugol's therapy has been graded ++ in those patients whose pulse rates fell, who gained in weight and who became calm and quiet, + when the improvement was not as marked, and ± when it was impossible to tell whether iodine

TABLE I  
*Patients with symptoms of definite hyperthyroidism*

Case number, sex and age	Blood iodine	Serum choles- terol	Basal meta- bolic rate	Symptoms of hyperthyroidism				Duration hyper- thyroid symptoms	Nutri- tion	Improvement		
				Thyroid enlargement	Tremor	Exoph- thalmos	Skin			After Lugol's, before thyroidectomy		After thyroid- ectomy
										Clinical symp- toms	Basal meta- bolic rate	Basal meta- bolic rate
	<i>gamma per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>per cent</i>					<i>months</i>			<i>per cent</i>	<i>per cent</i>
4342 F 46	7.5		+26	++ Nodular 4 years	0	0	0	216	N.	+	+12	+9
10184 M 41	7.7		+43	++	++	0	++	36	=	+	+28	+5
10493 F 32	8.6	150	+27	+++	+	++	0	1½	—	+	+1	—16
71934 F 50	8.5	175	+29	++	+	+	+	6	—	++	+10	+10
84098 M 32	17.9	84	+41	+++	+	++	+	24	—	++	+7	—9
A2590 F 29	7.0	131	+29	+	+	0	++	3	=	+	+17	
A84439 F 36	7.6	164	+41	++	+	0	+	5	=	++	+5	+1
A96720 M 25	9.2	132	+52	++	+	0	+++	6	—	+	+26	—1
A97068 F 42	8.3	142	+35	++	++	+	++	4	—	+	+21	—7
A99905 F 56	12.1		+41	+	0	0	++	3	—	+	+38	—5
B582 F 25	11.2	151	+52	++	++	0	++	2	N.	+	+20	+6
B693 F 33	21.9	99	+71	+++ Nodular	++	++	++	24	—	+	+55	—8
B832 F 26	13.6	103	+36	++	+	0	+	4	=	++	+8	+1
B933 F 30	12.0	177	+82	++	+	+	+	36	N.	+	+11	+16
B3260 F 26	14.2	170	+66	+	+	0	+	4	N.	++	+40	+6
B3296 F 36	9.7	135	+47	++	+	0	++	144	N.	+	+27	+6
B3842 F 24	9.6	91	+57	+++ Nodular	+	+	+	2	N.	++	+17	—4
B4123 F 24	15.0	106	+89	+++	++	++	++	40	—	+	+59	+14
B4507 F 61	7.4	183	+38	+++	+	0	+	4	N.	+	+18	+2

TABLE I (Continued)

Case number, sex and age	Blood iodine	Serum choles- terol	Basal meta- bolic rate	Symptoms of hyperthyroidism				Duration hyper- thyroid symptoms	Nutri- tion	Improvement		
				Thyroid enlargement	Tremor	Exoph- thalmos	Skin			After Lugol's, before thyroidectomy		After thyroid- ectomy
										Clinical symp- toms	Basal meta- bolic rate	Basal meta- bolic rate
	<i>gamma per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>per cent</i>					<i>months</i>			<i>per cent</i>	<i>per cent</i>
B4719 F 48	14.9	112	+54	++	++	+	+	6	—	+	+15	—4
B5149 F 43	8.2	179	+39	+	+	0	+	2 16 years recurrent	—	+	+15	—2
B5252 F 26	9.6	162	+37	++	++	0	++	7	N.	+	+13	+3
B5309 F 46	12.9	130	+54	++	++	0	++	3	=	+	+25	+1
B6138 F 41	9.5	146	+55	++	+	0	+	2	—	+	+36	—1
B6234 M 27	7.2	116	+68	++	++	+	+++	12	=	+	+21	+1
B7257 F 16	16.5	109	+86	+++	++	0	++	7	—	+	+45	+18
B7329 F 41	6.4	125	+27	+ Nodular	+	0	+	36	ob.	+	+9	—10
B8749 F 40	11.6		+68	++	++	+	+	12	~	+	+44	+11
1 F 58	7.8	158	+12	++ 15 years	+	0	0	9	=	+		
99948 F 33	7.2	183	+24	+ Nodular	+	0	+	12	N.	+	+4	
B4777* F 56	9.2	95	+52	+ Nodular 7 years	+	0	++	24	=	+	+26	

therapy had been of benefit. A blank space indicates that there were no data because Lugol's therapy was not tried or because there was no operation. It was impossible to state whether the operated patients were permanently improved since all these patients have been studied within the last 15 months.

Enlargement of the thyroid has been graded as + when the gland was slightly palpable, ++ when large, and +++ when large with bruit. Sub indicates a substernal thyroid. Under tremor + indicates a perceptible fine tremor of the extended tongue or fingers of the hand, ++ an easily recognizable tremor. Any perceptible exophthalmos or lid lag has been listed as +. Where the signs of exophthalmos were marked, ++ has

been used. Under skin have been included flushing, sweating, and satiny texture. Different degrees of flushing and sweating have been described as + to +++. Nutrition has been rated as N. when normal or average, — when approximately 10 per cent of weight has been lost, and = when emaciation was more severe. For people who remained distinctly well nourished, + has been used, and ob. indicates overweight of an extreme degree in which the distribution of fat was eccentric.

The chart presents diagrammatically the iodine values obtained by duplicate analyses of the blood of 26 normal subjects, of the 31 clear-cut hyperthyroid patients of Table I and of the patients in Tables II and III. For comparison, values from 6 subjects with nodular goiter

TABLE II  
*Patients with complications in whom hyperthyroidism was doubtful*

Case number, sex and age	Blood iodine	Serum cholest- erol	Basal meta- bolic rate	Symptoms of hyperthyroidism				Duration of symp- toms	Nutri- tion	Improvement		
				Thyroid enlargement	Tremor	Exoph- thalmos	Skin			After Lugol's, before thyroidectomy		After thyroidec- tomy
										Clinical symp- toms	Basal meta- bolic rate	Basal meta- bolic rate
	<i>gamma per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>per cent</i>					<i>months</i>			<i>per cent</i>	<i>per cent</i>
84736* F 52	6.4	113	+92	+ Nodular 10 years	0	0	+	120	=	±		
66560 F 51	5.9	243	+16	+	0	0	+	4	++			
B3513 F 58	7.4	105	+22	+	++	0	+++	10	=	±	+20	
B10541 F 61	6.3	149	+26	+ Sub.	+	0	++	24	N.	±	+20	
45153* F 53	8.9	233	+32	+	0	0	+	7	N.	+	+19	
91064* F 66	7.6	218	+23	? Sub.	+	0	++	36	N.	±	+6	
B4976 * F 35	7.6	154	+11	0	0	0	+	12	=			

and without signs of thyroid overactivity (Table IV), and from 7 patients with myxedema have been included. The narrow normal range is clearly indicated.

## RESULTS

All the patients in Table I had definite symptoms of hyperthyroidism. With the exception of 4342, 10493, 71934, A2590, B7329, 1, and 99948 the basal metabolic rates before Lugol's were between plus 35 and plus 89 per cent. After Lugol's all the patients had clinical improvement and the basal metabolic rates fell in all except Case 1. The thyroid was large in all but 7 cases, A2590, A99905, B3260, B5149, B7329, 99948 and B4777, in which it was distinctly palpable. In 7 cases there was a definite bruit as has been noted by +++ in the thyroid enlargement column. Clinically, hyperplasia of the thyroid was diagnosed in 25 patients, and nodular goiter with thyroid overactivity in 6. Tremor was a characteristic symptom of all the patients except A99905 and 4342. Satiny texture of the skin, typical of hyperthyroid patients, was noted in all

31 subjects with the exception of 1, 4342, and 10493. In 21 patients the nutrition was poor and there had been a loss of at least 10 per cent of the individual's usual body weight.

The 7 patients, with the exception of 84736 in Table II, had symptoms of hyperthyroidism which were not as outstanding as those of the patients in Table I. This can be seen easily in the table under "Symptoms of hyperthyroidism." Only 2 had basal metabolic rates above plus 30 per cent. None of the patients had a thyroidectomy.

The patients in Table III did not have definite symptoms of hyperthyroidism. Their basal metabolic rates varied between minus 10 and plus 25 per cent. Vasomotor and emotional instability was pronounced and the possibility of hyperthyroidism was considered.

The blood iodines of all the 31 patients in Table I were above the normal range. This is shown graphically in Figure 1. There was no overlapping with the normal values. The blood iodines ranged from 6.4 to 21.9 with an average of 10.6 gamma per 100 cc., while 2.4 to 4.2 with

TABLE III

*Patients in whom hyperthyroidism was considered because of nervous symptoms or vasomotor instability*

Case number, sex and age	Blood iodine	Serum cholesterol	Basal metabolic rate	Symptoms of hyperthyroidism				Duration of symptoms	Improvement			
				Thyroid enlargement	Tremor	Exophthalmos	Skin		After Lugol's, before thyroidectomy		After thyroidectomy	
									Clinical symptoms	Basal metabolic ratio	Basal metabolic ratio	Basal metabolic ratio
	gamma per 100 cc.	mgm. per 100 cc.	per cent					months		per cent	per cent	per cent
53949* M 38	4.8	174	+25	++	+	+	+	24	N	±	+16	+3
A29570* F 41	3.6	230	+5	+	+	0	0	84	N			
B5465* F 43	4.9	192	-3	+	0	0	0	18	N	±	-8	
B5469* F 27	4.2	241	-10	0	0	0	0	180	=			

an average of 3.1 gamma per 100 cc. represents the range of our present series of 26 normal subjects.

The height of the blood iodine was not related to the duration of symptoms. For example, 2 of the highest values, 17.9 (84098) and 15 gamma per cent (B4123) occurred in patients who had had symptoms for 24 and 40 months. The average of the blood iodines of the 18 patients with symptoms lasting less than 12 months was 10.5, and of the 13 patients with symptoms for 12 months or longer was 10.9 gamma per cent.

The 7 patients in Table II, in whom hyperthyroidism was doubtful because of complications, also had elevated blood iodines ranging from 5.9 to 8.9 gamma per cent. Case 84736 has been diagnosed as a hyperthyroid for 10 years. She has had pernicious anemia and has refused thyroidectomy. Cases 66560, B3513 and B10541, with blood iodines of only 5.9, 7.4, and 6.3 gamma per cent, had symptoms suggestive of early hyperthyroidism. The significance of the elevation in blood iodine of B4976 and 91064 is not clear. These studies on patients in Table II show that the blood iodine may be elevated without the accompaniment of definite hyperthyroidism.

The patients in Table III did not have definite symptoms of hyperthyroidism. Case 53949, with a blood iodine of 4.8 gamma per cent—not ap-

TABLE IV

*Patients with nodular goiter but without definite signs of thyroid activity*

Case number, sex and age	Blood iodine	Serum cholesterol	Basal metabolic rate	Symptoms of hyperthyroidism				Nutrition	Improvement		
				Thyroid enlargement	Tremor	Exophthalmos	Skin		Clinical symptoms	After Lugol's, before thyroid-ectomy	After thyroid-ectomy
										Basal meta-bolic rate	Basal metabolic rate
	gamma per 100 cc.	mgm. per 100 cc.	per cent							per cent	per cent
2 F 26	1.8		-2	++ Nodular	0	0	0	N.	±		
4404 M 21	2.9		+15	Nodular 16 years	0	0	0	N.			
A60174 F 43	3.6		+16	Calcified Nodule	0	0	+	-			
B8554 F 56	3.9	217	+31	++ Nodular	+	0	+	ob.	±	+28	
P2439 F 37	3.0	205	-7	++ Nodular 12 years	+	0	0	+			
P2495 F 76	4.9	305	+28	+ Nodular 62 years	++	0	0	N.	±		

preciably above the normal range—had a thyroidectomy because slight overactivity of the thyroid might be aggravating cardiac decompensation. He did not improve after thyroidectomy.

The 6 patients in Table IV, who had nodular goiters without symptoms of thyroid overactivity, had blood iodines within or not significantly dif-

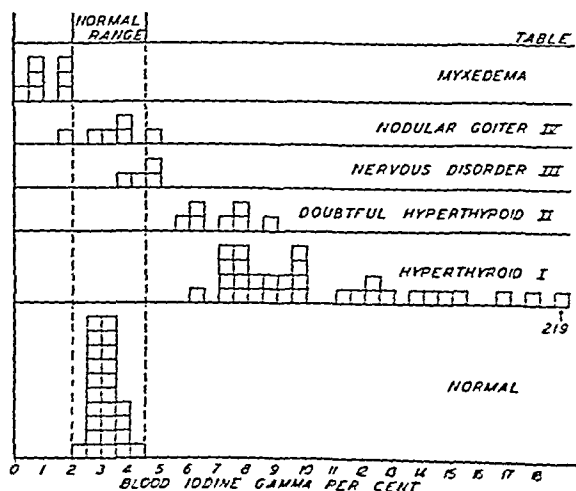


FIG. 1. A COMPARISON OF BLOOD IODINE VALUES IN: NORMAL SUBJECTS, AND IN PATIENTS WITH HYPERTHYROIDISM, MYXEDEMA, AND NERVOUS DISORDERS

ferent from the normal range. This is shown clearly in Figure 1.

In Figure 1 it can be seen that the blood iodine of each of the 7 myxedema patients was below the normal range. The values varied from 0.3 to 1.7 gamma per cent. All these patients had classical symptoms of myxedema. Two developed symptoms some years after thyroidectomy when thyroid medication was stopped temporarily. These patients will be described more fully in an article to be published.

Only 16 of the 27 definite hyperthyroid patients in Table I, who had serum cholesterol determinations, had cholesterols below the minimum of the normal range—150 milligrams per cent. For this reason, blood iodine is a more useful measure of thyroid overactivity than serum cholesterol.

#### DISCUSSION

The finding that the blood iodines of all 31 definite hyperthyroid patients were 1.5 to 5 times the highest normal value is at variance with previous observations. This is due partially to the fact that the iodines of our 26 normal subjects all fell within the narrow range of 2.4 to 4.2 gamma per cent. This range is wider than that previously published (1) owing to the addition of 6 normal subjects to our series. Five of the euthyroid individuals included in Figure 1 have been studied repeatedly at various times of the year. Only one determination on each of these individuals is included in the figure, but at no time of the year did their blood iodines exceed the normal range given above. It seems, therefore, that this normal range will not be increased by fluctuations which might be related to the season of the year. Our normal values agree closely with those of Baumann and Metzger (2) who found blood iodines ranging from 2.3 to 4.4 in 10 normal male and 10 normal female subjects. The lack of overlapping with the normal figures in the present study makes it improbable that a normal blood iodine will be found in a larger series of patients with hyperthyroidism. In view of the reports of Perkin and his associates (10, 11, 12) that normal blood iodine values are frequently found in patients with long-standing symptoms of hyperthyroidism, the duration of symptoms was carefully evaluated. It has been noted in the results that the average blood iodine of patients with symptoms which had persisted

from 12 to 216 months was practically the same as the average blood iodine of patients with symptoms of less than a year's duration.

Salter in his recent comprehensive monograph (13) and Davis, Curtis and Cole (14) have discussed the older published values showing large variations in blood iodine of hyperthyroid patients. Salter has described differences in values attributable to the region where the subject lives. Perkin and Lahey (12), Curtis and Puppel (15), as well as Salter, have pointed out that refinements in chemical techniques have resulted in lower blood iodine values of both normal and hyperthyroid subjects. Since the earlier values have been adequately discussed, our data need to be compared only with those published in 1940 by 3 different groups of workers.

The average blood iodines of normal and of hyperthyroid subjects reported by Klassen, Bierbaum and Curtis (16) agree with ours, but their normal range extends higher and their hyperthyroid range lower than ours. Thus, their normal values vary from 3.1 to 5.6 with an average of 4.0 gamma per cent, while their hyperthyroid values vary from 5.5 to 15.7 with an average of 10.0 gamma per cent. The analyses of Klassen, Bierbaum and Curtis were made on one aliquot of blood. A single analysis may at times be slightly high or low, and this may explain the slight overlapping of normal and hyperthyroid values which they have found. Our figures are the average of duplicate determinations which usually did not differ by more than 0.3 gamma per cent. Occasionally, when our duplicates failed to check within 0.5 gamma per cent, a triplicate analysis was made.

The results of Klassen, Bierbaum and Curtis agree more satisfactorily with the data presented in this paper than the findings of Turner, DeLamater and Province, and of Perkin. Turner, DeLamater and Province (17) found a wider range of 3.5 to 10.4 gamma per cent in 20 normal males and 20 normal females and a higher normal average of 6.3 gamma per 100 cc. These investigators made duplicate analyses, using the method of Trevorrow and Fashena. Their normal values are in close agreement with Fashena's (18) normal range of 3.0 to 12.0 and average of 6.6 gamma per cent in 79 children 2 days to 13 years in age. For some reason Fashena's method gives higher

normal values than have been found by Curtis and his associates (4, 15, 16), by Grauer and Saier (3) and by Baumann and Metzger (2). The results of the latter workers were cited above. Turner, DeLamater and Province found a marked variation in the blood iodine of normal subjects and a wide range for their 20 hyperthyroid patients of 6.7 to 38.5 gamma per cent. The blood iodines of 6 of the 20 thyroid patients fell within the normal range. This overlapping is not surprising considering the wide fluctuations between the minimum and maximum normal and hyperthyroid values.

Perkin and Lahey (12) have found an even greater overlapping of normal and hyperthyroid values. They have found persons with no clinical evidence of thyrotoxicosis whose iodines ranged between 2 and 15 gamma per cent, although they have accepted 10 gamma per hundred cc. as the upper limit of normal. About 34 per cent of their hyperthyroid patients had blood iodines of less than 10 gamma per cent. Their method of analysis depends on alkaline oxidation of the blood with alcoholic extraction of iodine compounds from the charred mass. This method has been criticized for the difficulties in technique and for erroneously high values (2, 15). Some of the enormous overlapping of normal and hyperthyroid values can therefore be attributed to technical difficulties in the analysis. In spite of wide variations in data, it can be concluded from all published figures that an elevation of blood iodine above the normal range is usual in hyperthyroid patients.

A high blood iodine, on the other hand, does not always connote definite hyperthyroidism. This was emphasized with respect to the patients in Table II. As has been stated, these patients had not had iodine solutions as contrast media for x-ray diagnosis. This is important because diodrast may not be completely eliminated for days, iso-iodeikon (Graham-Cole test) for months and lipiodol for years. Of the 3 patients, 45153, 91064 and B4976, who had blood iodines of 8.9, 7.6, and 7.6 gamma per cent, the first 2 had cardiac enlargement and arteriosclerosis. Case 45153 also had diabetes which required insulin. All 3 had vasomotor or emotional instability. Their symptoms seemed to be more closely related to nervous system disorders than thyroid overactivity. Patients like these present a problem in clinical diag-

nosis and it was hoped that the blood iodine would be a useful criterion to help distinguish them. However, all the patients in Table III had similar symptoms with blood iodines which were normal or only insignificantly elevated. Conclusions cannot be formulated until further studies of these and similar patients are completed. No attempt is made here to review the literature on this subject since Salter (13) has discussed at length the action of the thyroid hormone on the nervous system and the action of the nervous system on the thyroid.

The results on the 7 myxedematous patients were as clear-cut as those on the patients with hyperthyroidism. The highest blood iodine of 1.7 gamma per cent was well below the minimum normal value of 2.4 gamma per cent.

That the levels of blood iodine and serum cholesterol in thyroiditis are similar to those observed in definite hyperthyroidism was observed by Turner and his associates (17) and also noted by us in one female patient. Her blood iodine was 8.5 gamma per cent. Her basal metabolic rate was minus 5 per cent, and her serum cholesterol was 126 milligrams per cent, but she had no exophthalmos, motor restlessness or appearance of the skin typical of hyperthyroidism. Turner, DeLamater and Province reported elevated blood iodines of 11.2 and 11.3 gamma per cent in 2 cases of thyroiditis. The serum cholesterols were 124 and 141 milligrams per cent. These values approach the low levels in clear-cut hyperthyroidism (19).

#### CONCLUSIONS

The level of blood iodine has been found to be closely related to thyroid activity.

In 31 patients with hyperthyroidism the blood iodine ranged between 6.4 and 21.9 gamma per cent.

In 7 patients with myxedema it varied from 0.3 to 1.7 gamma per cent.

There was no overlapping with the normal range of 2.4 to 4.2 gamma per cent in 26 euthyroid subjects.

Six patients with nodular goiters but without symptoms of thyroid overactivity had blood iodines which did not differ significantly from normal.

It has been concluded that elevated blood iodine values are invariably found in hyperthyroid patients. However, certain patients without mani-



fest hyperthyroidism may also have an elevated blood iodine. Further study of such patients is indicated.

It would have been impossible to collect this material if it had not been for the clinical services of Dr. Paul Lavietes, Dr. Alexander Winkler, and Dr. Kalmen A. Klinghoffer under whose care were many of the patients.

#### PROTOCOLS

B4777, female, aged 56. This patient had chronic myelogenous leukemia with a white count between 750 and 3000. The basal metabolic rate was plus 52 per cent, serum cholesterol 95 milligrams per cent, and blood iodine 9.2 gamma per cent. The response in basal metabolic rate to Lugol's confirmed the diagnosis of hyperthyroidism, but no operation was performed because of complications.

84736, female, aged 52. This patient had developed severe pernicious anemia 11 years previously. She also had slight exophthalmos, tremor, a nodule in the thyroid and a basal metabolic rate of plus 50 per cent at that time. She responded quite well to treatment with liver extract and iodine. After 2 years iodine was omitted and the patient did fairly well for 3 years. Then symptoms of hyperthyroidism returned and the basal metabolic rate was plus 31 per cent. Lugol's administration was followed by a remission. Iodine was stopped in June 1939 and she remained asymptomatic until June 1940 when palpitation, tremor and loss of weight returned. Operation was again advised and refused, as it had been many times before. In addition to hyperthyroid symptoms, the liver and spleen had become markedly enlarged. Iodine treatment was given from June to August 15, 1940, and then omitted. In October, the basal metabolic rate was plus 92 per cent, serum cholesterol 113 milligrams per cent, and blood iodine 6.4 gamma per cent. Subsequent return to iodine therapy was followed by modest improvement in 2 to 3 months. For the past 10 years the patient has required regular doses of liver or reticulogen to prevent return of anemia.

45153, female, aged 53. Hypertension had developed following toxemia of pregnancy 27 years previously. Diabetes mellitus and arteriosclerotic cardiovascular disease had been noted 10 years ago. The patient took insulin and digitalis so irregularly that she had repeated attacks of cardiac decompensation from which she recovered when adequate therapy with digitalis, insulin and diet was restored. Emotional instability, multiple complaints, unsteadiness of hands, flushing of skin, sweating, weight loss and diarrhea had become increasingly prominent during the last 2 years. On January 15, 1940, the basal metabolic rate was plus 32, serum cholesterol 233 milligrams per cent, and blood iodine 8.9 gamma per cent. Lugol's therapy was followed by considerable improvement in all symptoms in 2 months.

91064, female, aged 66. For the last 10 years recurrent and increasingly severe attacks of decompensation had been associated with arteriosclerotic cardiovascular

disease. On the last hospital admission the patient had congestive heart failure with auricular fibrillation and anasarca. At this time she also had nervousness, tremor of hands and profuse sweating, although the thyroid was not enlarged. The basal metabolic rate was plus 23 per cent, serum cholesterol 218 milligrams per cent and blood iodine 7.6 gamma per cent. She died in congestive failure 5 weeks later.

B4976, female, aged 35. This patient had had multiple complaints of anxiety, palpitation, anorexia, vomiting and loss of 60 pounds during the previous year. After KI therapy from January 17 to 31, 1940, she was reported to have improved. In May, 1940, the patient was well nourished, had moist skin and extensive leukoderma. She looked exhausted and depressed and her movements were slow, but she was also tense and irritable. On May 9, 1940, the basal metabolic rate was plus 11, serum cholesterol 154 milligrams per cent and blood iodine 7.6 gamma per cent, but other signs of hyperthyroidism were lacking. While anxiety and depression were the outstanding features, overactivity of the thyroid was not entirely ruled out. Iodine was not given and she was referred to psychiatrists for treatment.

53949, male, aged 38. Following osteomyelitis of the jaw 3 years previously, this patient had successively developed hypertension, albuminuria, restlessness, irritability and a basal metabolic rate of plus 25. On admission, June 19, 1940, he was excitable, talkative, well nourished, and had prominent eyes, enlarged thyroid, hypertensive arteriosclerosis and an enlarged heart with slight decompensation. The serum cholesterol was 174 milligrams per cent and blood iodine 4.8 gamma per cent. Lugol's administration produced slight improvement. Subtotal thyroidectomy was followed by little change in symptoms and 2 months later the patient was unimproved although the basal metabolic rate was plus 3 per cent.

A29870, female, aged 41. This restless, apprehensive patient had had repeated illnesses characterized by weakness, insomnia, "palpitation," emotional upset, weight loss and varying physical complaints for 15 to 20 years. During these illnesses the basal metabolic rate had varied between plus 25 and plus 5 per cent. Response to iodine administration was uncertain because remissions also occurred without iodine. The last attack began in April, 1940, with pain in the epigastrium followed by loss of appetite and weight. On admission, September 16, 1940, the multiple complaints and emotional lability were striking. There was sweating and inconstant tremor of hands. The basal metabolic rate was plus 5 per cent, blood iodine 3.6 gamma per cent and serum cholesterol 230 milligrams per cent. Rest in bed without iodine resulted in marked improvement in symptoms. Emotional instability and spells of depression remained, although the tremor had disappeared. As all symptoms were referable to nervous system disorders, and other signs of thyroid disorder were lacking, the patient was referred to psychiatry for treatment.

B5465, female, aged 43. Fifteen months previously nervousness, which had been noticed for years, had become more marked and, subsequently, palpitation, prom-

inence of eyes, tremulousness and dyspnoea appeared. In addition, the menses became irregular and she lost weight. On admission the patient was tense and apprehensive and had a slight tremor of the hands. Her blood pressure was 140/98 and the pulse 140. By the next day most of her symptoms had subsided, and little but the vasomotor instability could be elicited. The thyroid was palpable, the right lobe being larger than the left. On May 28, 1940, the basal metabolic rate was minus 3 per cent, serum cholesterol 192 milligrams per cent and blood iodine 4.9 gamma per cent. Two weeks of treatment with Lugol's therapy and bed rest did not relieve vasomotor lability or complaints of nervousness. The diagnosis was former hyperthyroidism in remission and beginning menopause.

B5469, female, aged 27. Severe headaches, fainting spells, narcolepsy and intermittent bouts of fever had developed after the patient had broken her nose 15 years previously. In addition, she had had sinusitis and mastoiditis which had been relieved by mastoidectomy 14 years ago. Later, occasional attacks of hives and edema had occurred. All these symptoms continued, being interrupted by partial remissions lasting a few months to 3 years. In the previous 2 to 3 years, bouts of fever, nausea, vomiting, and loss of 55 pounds had occurred. The patient was poorly nourished and dehydrated on admission, but fever of 101° subsided in 2 days. On June 4, 1940, the basal metabolic rate was minus 10 per cent, serum cholesterol 241 milligrams per cent and blood iodine 4.2 gamma per cent. Headaches and narcolepsy continued. It was concluded that the symptoms were probably due to a hypothalamic lesion of unknown etiology.

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# LUNG VOLUME UNDER SURGICAL ANAESTHESIA: THE EFFECT OF AVERTIN ON THE SUBTIDAL AIR<sup>1</sup>

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(Received for publication March 11, 1941)

Clinical and experimental evidence indicates that pulmonary hypoventilation and a decrease in pulmonary volume are fairly constant sequelae of operations performed in the abdominal cavity. Churchill and McNeill (1) observed a marked reduction in vital capacity following laparotomy. Beecher (2) and others have demonstrated that upper abdominal surgery causes a sharper reduction in vital capacity than lower abdominal types of operations, and that the reduction is greater for any given procedure in males than in females. Muller, Overholt and Pendergrass (3) found a significant rise in the level of the diaphragm following laparotomy and considered this a primary effect of the pneumoperitoneum, rather than a secondary effect of changes in the lung itself. Beecher (4) first studied the effect of laparotomy on the subtidal lung volume.<sup>2</sup> He demonstrated an average postoperative decrease of about 20 per cent, the drop becoming maximal on the fourth postoperative day, and returning toward the preoperative values during the second week. This significant study has suggested to us the necessity of noting the effect of surgical anaesthesia *alone* on the subtidal lung volume before factors incident to surgical manipulation enter the picture.

## MATERIALS AND METHODS

The use of an inhalation anaesthetic precludes the employment of any alveolar gas dilution technic for determining lung volume, such as that of Christie (5) which is the best available. However, an anaesthetic agent such as avertin (tribromethanol) which is administered rectally and not excreted at all through the respiratory tract (6) lends itself admirably to this study.

A group of 17 preoperative surgical cases from the general surgical and gynecological services of the New Haven Hospital was selected. When the choice of anaesthesia was known to be basal avertin, the past respira-

tory history of each case was carefully reviewed; any patient having recent or chronic symptoms was rejected, as was any subject with abnormal physical findings in the chest.

Preoperative control determinations of the subtidal volume were carried out according to the technic of Christie (5) sometimes in the post-absorption state on the day prior to operation, but more frequently on the morning of operation before any sedation had been given. Determinations of the subtidal volume were made in duplicate, at least, after a trial run to accustom the patient to breathing in a closed spirometer circuit. Particular care was taken to posture the patient in the supine horizontal position, with a single pillow under the head, and arms relaxed at the sides.

The patient was then returned to his room; a preliminary medication of morphine sulphate (grains  $\frac{1}{8}$  or  $\frac{1}{4}$ ) and atropine sulfate (grains  $\frac{1}{450}$  or  $\frac{1}{400}$ ) was given except in 2 cases (Cases 9 and 16) in which it was deliberately omitted for control purposes. Avertin in amylene hydrate was then administered rectally, the dosage and time being exactly noted. The usual dosage was equivalent to 70 to 90 mgm. per kilo of body weight. When the patient was asleep he was moved again to the laboratory and carefully adjusted to the above described position. A wire pharyngeal airway was inserted to support the tongue, and to avoid expiratory obstruction which may cause a considerable increase in subtidal volume (7). The jaws were supported by the anaesthetist, and the patient was connected with the spirometer, the pharyngeal airway being accommodated into the rubber mouthpiece and the lips carefully sealed with adhesive to prevent leakage. At least one, and if time and the surgeons' patience permitted, two determinations were made; the patient was then sent to the operating room, and the indicated procedure was carried out with supplementary local or inhalation anaesthesia. Before the patient was discharged from the hospital repeat determinations were made in 11 of the 17 cases.

## RESULTS

The various data relevant to the 17 cases are best presented by the accompanying chart (Table I). It will be noted that the series included 7 males and 10 females with a wide selection of non-thoracic complaints.

Fourteen of the 17 cases showed a decrease in subtidal lung volume under the influence of avertin anaesthesia. The decrease in these 14 cases ranged

<sup>1</sup> This study was aided by a grant from the Fluid Research Fund of the Yale University School of Medicine.

<sup>2</sup> The subtidal lung volume is here defined as the air content of the respiratory tract at the end of a normal tidal expiration.

TABLE I  
Summary of cases

TABLE I Summary of cases																	
Type of operation	Height inches	Weight pounds	Surface area square meters	Avertin dosage cc.	Drop in systolic blood pressure mm.	Type of induction	Preoperative			Avertin anaesthesia				Day	Mean subtidal cc.	Deviation	
							Subtidal mean cc.	Deviation	Minutes elapsed	Subtidal cc.	Change cc.	Decrease per cent	Pulmonary complications				
cholecystitis	60	160	1.70	4.9	68	Rapid 5 minutes	1790	± 10	25	1520	- 270	17	13	2060	± 40		
	66	145	1.75	5.7	75	Good 8 minutes	3040	± 70	45	1490	- 300	14					
Gastroenterostomy	92	130	3.8	0	0	Good 8 minutes	1880	± 140	33	1580	- 300	16	7	1840	± 50		
	110	155	5.6	10	0	Poor sec- ond dose	3680	± 160	45	1800	- 80	15	13	3530	± 240		
Hysterectomy	152	165	5.4	0	0	Good 10 minutes	3600	± 60	17	3440	- 240	11					
	98	140	4.2	0	0	Slow 20 minutes	2360	± 110	73	3120	- 560	36					
Hysterotomy	136	160	5.8	0	0	Slow	1860	± 50	24	3190	- 410	0					
	153	165	6.0	20	Good 8 minutes	1950	± 120	35	1500	- 860	36	14	2600	± 100			
in pregnancy	62½	136	4.2	0	0	Incom- plete	1860	± 50	37	1880	+ 20	0					
	153	165	6.0	20	Good 8 minutes	1950	± 120	21	1640	- 310	22	13	2090	± 140			

TABLE 1—Continued

Case number	Sex	Age	Diagnosis	Type of operation	Height inches	Weight pounds	Surface area square meters	Avertin dosage cc.	Drop in systolic blood pressure mm.	Type of induction	Subtidal lung volume									
											Preoperative		Avertin anaesthesia				Postoperative			
											Subtidal mean cc.	Deviation	Minutes elapsed	Subtidal cc.	Change cc.	Decrease per cent	Day	Mean subtidal cc.	Deviation	
9	M	53	Chronic cholecystitis	Cholecystectomy	65½	127	1.60	5.2	10	Slow ?	3330		20 40	3070 2120	— 260 — 1210	36	Pulmonary complications			
10	F	27	Ovarian cysts	Salpingo-oophorectomy	58½	118	1.45	4.7	20	Good 5 minutes	2350	± 10	21	2170	— 180	7	13	2150	± 80	
11	M	21	Deformity of hip	Osteotomy	63	117	1.50	5.4	0	Good 8 minutes	3190	± 50	22 37	2620 2720	— 570 — 470	18		2590	± 140	
12	M	42	Inguinal hernia	Herniorrhaphy	71½	159	1.90	6.9	28	Incom- plete	4040	± 140	22	4180	+ 140	0				
13	M	11	Inguinal hernia	Herniorrhaphy	59	86	1.25	4.5	18	Slow 15 minutes	1700	± 90	26 42	1670 1740	— 30 + 40	0				
14	F	27	Uterine myoma	Hysterectomy	65½	175	1.90	6.2	10	Good 5 minutes	3390	—	23 42	2840 3130	— 550 — 260	16	11	2750	± 10	
15	M	38	Ventral hernia	Herniorrhaphy	70	218	2.10	7.6	0	Good 8 minutes	2550	± 30	23 37	1770 1720	— 780 — 830	32	14 16	2350 2310	0 ± 40	
16	F	54	Chronic cholecystitis	Cholecystectomy	64	169	1.80	6.5	24	Good 5 minutes	2100	± 60	21	1810	— 290	14	13	1700	± 30	
17	F	32	Cystocele, etc.	Perineal repair	62½	195	1.85	6.3	0	Fair 5 minutes	1430	± 100	35 52	1180 1190	— 250 — 240	17	13	1700	± 50	

TABLE I  
Summary of cases

Case number	Sex	Age	Diagnosis	Type of operation	Height <i>inches</i>	Weight <i>pounds</i>	Surface area <i>square meters</i>	Avertin dosage <i>cc.</i>	Drop in systolic blood pressure <i>mm.</i>	Type of induction	Subtidal lung volume									
											Preoperative		Avertin anaesthesia				Postoperative			
											Subtidal mean <i>cc.</i>	Deviation	Minutes elapsed	Subtidal <i>cc.</i>	Change <i>cc.</i>	Decrease <i>per cent</i>	Day	Mean subtidal <i>cc.</i>	Deviation	
1	F	58	Chronic cholecystitis	Cholecystectomy	60	160	1.70	4.9	68	Rapid 5 minutes	1790	$\pm 10$	25 45	1520 1490	- 270 - 300	17	13	2060	$\pm 40$	
2	M	55	Peptic ulcer	Gastroenterostomy	66	145	1.75	5.7	75	Good 8 minutes	3040	$\pm 70$	15 39	3050 2600	+ 10 - 440	14				
3	F	22	Chronic mastitis	Simple mastectomy	59	92	1.30	3.8 1.4	0	Poor sec- ond dose	1880	$\pm 140$	33 45	1580 1800	- 300 - 80	16	7	1840	$\pm 50$	
4	M	19	Subsiding appendicitis	Appendectomy	65½	110	1.55	5.6	10	Good 10 minutes	3680	$\pm 160$	17 73	3440 3120	- 240 - 560	15	13	3530	$\pm 240$	
5	F	48	Chronic cholecystitis	Cholecystectomy	61½	152	1.65	5.4	0	Slow 20 minutes	3600	$\pm 60$	24	3190	- 410	11	Pulmonary complications			
6	F	33	Myoma uteri	Hysterectomy	63½	98	1.40	4.2 0.6	0	Slow	2360	$\pm 110$	35	1500	- 860	36	14	2600	$\pm 100$	
7	F	34	Toxemia pregnancy	Hysterotomy	62½	136	1.60	5.8	0	Incom- plete	1860	$\pm 50$	37	1880	+ 20	0				
8	F	34	Pelvic infectious disease	Hysterectomy, etc.	61	153	1.65	6.0	20	Good 8 minutes	1950	$\pm 120$	21 36	1640 1510	- 310 - 440	22	13	2090	$\pm 140$	

TABLE I—Continued

Case number	Sex	Age	Diagnosis	Type of operation	Height	Weight	Surface area	Avertin dosage	Drop in systolic blood pressure	Type of induction	Subtidal lung volume									
											Preoperative		Avertin anaesthesia				Postoperative			
											Subtidal mean	Deviation	Minutes elapsed	Subtidal	Change	Decrease	Day	Mean subtidal	Deviation	
					inches	pounds	square meters	cc.	mm.		cc.		20 40	cc. 2120	cc. — 260 — 1210	per cent 36		cc.		
9	M	53	Chronic cholecystitis	Cholecystectomy	65½	127	1.60	5.2	10	Slow ?	3330									
10	F	27	Ovarian cysts	Salpingo-oophorectomy	58½	118	1.45	4.7	20	Good 5 minutes	2350 ± 10		21	2170	— 180	7	13	2150	± 80	
11	M	21	Deformity of hip	Osteotomy	63	117	1.50	5.4	0	Good 8 minutes	3190 ± 50		22 37	2620 2720	— 570 — 470	18		2590	± 140	
12	M	42	Inguinal hernia	Herniorrhaphy	71½	159	1.90	6.9	28	Incom- plete	4040 ± 140		22	4180	+ 140	0				
13	M	11	Inguinal hernia	Herniorrhaphy	59	86	1.25	4.5	18	Slow 15 minutes	1700 ± 90		26 42	1670 1740	— 30 + 40	0				
14	F	27	Uterine myoma	Hysterectomy	65½	175	1.90	6.2	10	Good 5 minutes	3390 —		23 42	2840 3130	— 550 — 260	16	11	2750	± 10	
15	M	38	Ventral hernia	Herniorrhaphy	70	218	2.10	7.6	0	Good 8 minutes	2550 ± 30		23 37	1770 1720	— 780 — 830	32	14 16	2350 2310	0 ± 40	
16	F	5½	Chronic cholecystitis	Cholecystectomy	64	169	1.80	6.5	24	Good 5 minutes	2100 ± 60		21	1810	— 290	14	13	1700	± 30	
17	F	32	Cystocele, etc.	Perineal repair	62½	195	1.85	6.3	0	Fair 5 minutes	1430 ± 100		35 52	1180 1190	— 250 — 240	17	13	1700	± 50	



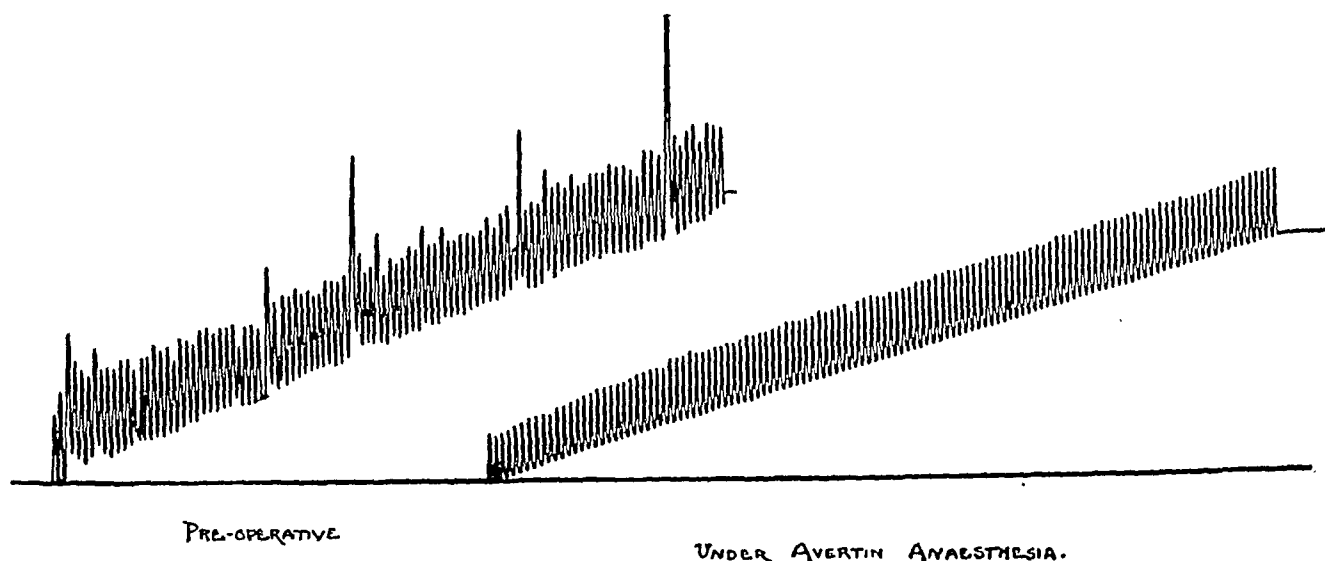


FIG. 1. SPIROMETER TRACINGS BEFORE AND DURING AVERTIN ANAESTHESIA. CASE 1

from 180 to 1,210 cc. and averaged 510 cubic centimeters, or 19 per cent of the average subtidal volume. Of the 3 cases which failed to show any decrease, 2 (Cases 7 and 12) were never completely unconscious, reacting to sensory stimuli, moving and talking. The third case (Case 13) was very slow in induction; in 15 minutes he was sufficiently relaxed to permit introduction of a pharyngeal airway which was impossible in the other 2. No postoperative studies were made in these 3, nor in 3 others because of death in 1 and postoperative pulmonary complications in 2 (Cases 2, 5, and 9). Of the remaining 11 in which postoperative determinations were made at least once before discharge, the subtidal lung volume had returned to the preoperative level or above in 5, almost completely so in 3, and not at all in 3.

A marked regularity of respiration and smoothness in the spirometer tracings was noticed under avertin anaesthesia. There was a decrease in minute ventilation and oxygen consumption. These changes are well illustrated by Case 1 (Figure 1).

	Preoperative control	Avertin
Duration of breathing period	5½ minutes	7
Respiratory rate.....	18	17
Mean tidal.....	410 cc.	340 cc.
Minute volume.....	7380 cc. per minute	5780 cc. per minute
Oxygen consumption.....	259 cc. per minute	183 cc. per minute
* Ventilation equivalent....	2.8	3.1
Subtidal lung volume.....	1790 cc.	1500 cc.

\* Number of liters of air respired for each 100 cc. oxygen consumed.

## DISCUSSION

Expiration in the normal resting state is a passive phenomenon brought about by the elastic recoil of the lung and chest musculature, and aided by gravity and the weight of the abdominal viscera in some positions. The level at which expiration ceases is determined by a balance between the elasticity of the lung, and the resistance of the thoracic and abdominal wall. The prevailing muscle tone must therefore play a primary rôle in the establishment of the resting expiratory level, and likewise the subtidal lung volume. Anaesthesia of any type, if it reduces muscle tone, should reduce the air content of the lungs. Clinical observations now being completed in this laboratory (8) indicate that morphine sedation alone will reduce the subtidal volume in most subjects, but at a slower rate than avertin. One might predict that during natural sleep the same effect obtains and this has been observed. Spirometer tracings from one of our patients who unexpectedly but fortunately fell asleep during the course of a lung volume determination some months after pulmonary lobectomy (Figure 2) show a marked sagging of the expiratory level (to the extent of 500 cubic centimeters) with a rapid return to the original level when the patient was stimulated and awakened by a sudden noise.

Clinical experience with avertin indicates that the depth of anaesthesia and the degree of muscular relaxation are variable and unpredictable, depending on dosage, rate of absorption and excretion. There should therefore be a considerable variation

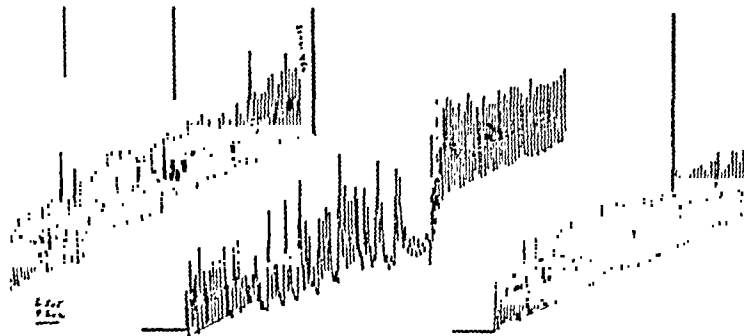


FIG. 2. SPIROMETER TRACINGS

Patient, undergoing routine post-lobectomy lung volume determinations, became drowsy and fell asleep during the course of the second (or middle) tracing. Note resumption of usual expiratory level when stimulated and fully awakened.

in the degree of lung volume decrease under the influence of this drug, and this seems to be the case. In 10 of the patients studied, it was possible to make two successive determinations under anaesthesia; it will be noted in Table I that, when the first determination was made less than 25 minutes after the rectal administration of the drug, the second determination was usually somewhat lower. This correlates with the fact that the maximal blood concentration (6 to 10 mgm. per cent) usually occurs about 30 minutes after administration, 85 per cent of the avertin having been absorbed at that time (6).

Of the 14 patients who demonstrated a decrease in subtidal lung volume under avertin, 5 were males, with an average decrease of 720 cc. and 9 females, with an average decrease of 390 cc. Whether this discrepancy has a bearing on the known preponderance of postoperative pulmonary complications in males cannot be definitely stated in view of the small number of cases involved.

Avertin exerts a strong depressant action on the medullary vasomotor centers. Five of the 17 cases showed an immediate drop of systolic blood pressure in excess of 20 millimeters. However, this group presented an average decrease of only 300 cc. in subtidal volume, as compared with 510 cc. for the whole group. It seems unlikely, therefore, that a primary vascular effect with redistribution of blood volume is responsible for the decrease in subtidal air.

#### SUMMARY AND CONCLUSIONS

1. A reduction in subtidal lung volume occurred in 14 of 17 subjects following the administration

of avertin (tribromethanol) in basal anaesthetic doses. In the remaining 3, anaesthesia was incomplete.

2. The maximum decrease was observed in the neighborhood of 30 minutes following administration of the drug when the circulating blood concentration of the drug is known to be highest.

3. The probable cause of this decrease is considered to be a diminution in muscle tone, with a consequent disturbance of the normal balance between the elastic lung and the supporting musculature of the thorax and abdomen.

4. Other evidences of respiratory depression are found and described.

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# THE RELATION OF HIGH AND LOW UREA CLEARANCES TO THE INULIN AND CREATININE CLEARANCES IN CHILDREN WITH THE NEPHROTIC SYNDROME

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During the past 6 years we have observed that, in children with the nephrotic syndrome, the urea clearance is not infrequently increased for periods of 1 or more months to 140 per cent or more of the average clearance of normal children of similar age and size. The phenomenon has occurred with the same frequency in both sexes. In a group of 33 nephrotic children all less than 10 years of age who were admitted to the Hospital, we have seen this type of elevation in 14 (or 42 per cent) of the patients. In 6 of the 14 patients the elevation has persisted for a period of at least 6 months; in 1 of our nephrotic children the urea clearance has been consistently elevated to between 200 and 300 per cent of normal for 6 years. These nephrotic children have been found by Farr (1) to show also a degree of lability of the urea clearance not noted in normal adult man (2) or, in our experience and in that of others, in children or adults (3) with decreased urea clearances. When the dietary protein was reduced from the optimum intake of 3 grams per kilo to 1 gram or less, the urea clearance showed a parallel fall. In contrast to our young children, only 2 out of a group of 54 nephrotic adults and children over 10 years of age observed in this Hospital have shown high clearances; these were aged 11 and 18 years.

The mechanism of this high urea clearance in nephrotic children has not been explained. In the present study we have sought to determine whether the increased urea clearance is accompanied by a similar increase in the inulin clearance, which is believed to equal the volume of the glomerular filtrate (4). We have also determined the ratios of inulin clearance to urea clearance and to creatinine clearance in these patients, and compared them with the same ratios in nephrotic children with diminished urea clearances, and in children who have recovered from acute nephritis

## *Patients studied and experimental procedures followed*

The patients studied in our experiments were 3 nephrotic children (R. Q., S. G., R. M.) with high urea clearances, 2 nephrotic children (J. C., S. W.) and 1 nephrotic adult (A. C.) with low urea clearances and, as controls with normal renal function, 2 children who had recovered from acute hemorrhagic Bright's disease. Of the 3 patients in the high clearance group, 1, R. M., had a urea clearance consistently elevated to above 140 per cent of normal; the other 2 patients had urea clearances always above 100 per cent of normal and frequently above 140 per cent. All the nephrotic patients exhibited proteinuria and hyperlipemia and had plasma albumin levels below 2.5 grams per 100 cc. Edema had been present previously in each case but was observed only in S. G. at the time of these experiments. Detailed laboratory and clinical data on 4 of these patients (R. Q., S. G., J. C., S. W.) have been published elsewhere (5, 6). For several months previous to these experiments all of the patients except B. D. and A. C. had been fed a diet which provided 3 grams of protein per kilogram of ideal body weight. The daily intake of sodium chloride was 1 to 1.5 grams.

All tests were performed under fasting conditions; the subjects were kept in bed during the clearance periods. Preceding and during the experiments, from 1 to 2 liters of water were administered orally to maintain an adequate flow of urine. The patients were not catheterized. After 2 or 3 control periods, each of approximately 1 hour's duration, during which specimens of urine and a single blood sample were obtained for the determination of urea and "endogenous" creatinine clearances, creatinine was administered orally. One hour later a single injection of inulin, prepared as a 10 per cent solution in 0.85 per cent sodium chloride solution,<sup>1</sup> was administered intravenously during the course of 15 to 20 minutes. The quantities of creatinine and inulin given varied in the individual experiments as shown in Tables I, II and III. Thirty to 60 minutes following the injection of inulin, urine collections were resumed for the determination of simultaneous inulin, "exogenous" creatinine, and urea clearances. The duration of these latter periods of urine collection varied usually from 30 to 60 minutes and was determined by the patients' desire to void. Venous blood samples were obtained at the beginning and end of each period.

<sup>1</sup> The 10 per cent solution of inulin in saline was purchased from the U. S. Standard Products Co., Woodworth, Wisconsin.

TABLE I  
Nephrotic patients with high urea clearances. Comparison of inulin, creatinine, and urea clearances

Subject	Period	Duration	Urine flow V $\ddagger$	Plasma levels			Urine levels			Clearances				Clearance ratios	
				Inulin	Creatinine	Urea N	Inulin	Creatinine	Urea N	Inulin	Endogenous creatinine	Exogenous creatinine	Urea	Exogenous creatinine: Inulin	Urea: Inulin
		minutes	cc. per minute	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	cc. plasma per minute	cc. plasma per minute	cc. plasma per minute	cc. plasma per minute		
R. Q. ♀ 8 years (V factor $\ddagger$ = 1.74) January 25, 1940	1	60	3.61			7.5*			230*				111*		
	2§	55	4.30			6.2*			177*				122*		
	3	60	6.68	11.6		6.2*	330		105*	190			113*		0.59*
	4	60	3.55	4.4		6.4*	280		192*	226			106*		0.47*
	Average									208			113		
Idem March 11, 1940	1	70	6.72		0.33	7.6		9.2	146		184		129		
	2	57	3.56					15.6	194		166		91		
	3	58	4.14					14.0	208		173		113		
	4¶	59	2.32		3.45	7.5		117.4	282				87		
	5	75	6.10		7.38	7.0		340.0	105			281	92		
	6	33	1.03	21.0	5.45	7.0	4300	2030.0	418	212		386	86	1.82	0.41
	7	32	2.40	14.6	4.55	6.5	1200	586.0	256	197		309	95	1.57	0.48
	8	41	3.84	10.4	3.78	6.5	450	220.0	128	166		223	75	1.34	0.45
	9	34	7.64	7.2	3.00	7.2	185	114.0	103	196		290	110	1.48	0.56
	Average									193	174	297	98		
S. G. ♂ 10 years (V factor $\ddagger$ = 1.75) February 26, 1940	1	114	2.01		0.28	9.1		24.7	418		177		92		
	2	61	4.70					11.7	202		197		104		
	3¶	60	3.04		2.30			295.0	262				88		
	4	60	6.25		3.67	8.9		216.0	123			390†	86		
	5	60	1.81	18.1	2.26	9.1	1520	420.0	346	152		336	72	2.21	0.47
	6	60	1.20	8.7	1.32	9.4	1380	440.0	468	190		370	77	1.95	0.41
	7††	62	0.62	5.6	0.95	12.8	1160	403.0	316	129††		263††	28††	2.04	0.22
	8	58	1.69	3.2	0.75	14.4	620	123.0	478	327		276	61	0.84	0.19
	Average									171	187	366	86		
R. M. ♂ 3 years (V factor $\ddagger$ = 2.75) April 16, 1940	1	55	2.99		†	7.1			323		†		136		
	2	60	11.10						76				119		
	3	58	6.55						116				103		
	4**	52	3.59		3.60			334.0	207			333†	105		
	5	50	7.05		11.96	6.7		634.0	101			373	105		
	6	35	10.80	51.5	11.12	6.7	1030	358.0	72	216		349	115	1.62	0.53
	7	30	6.96	20.0	9.40	6.6	760	400.0	95	265		296	100	1.12	0.38
	8	30	1.46	14.3	7.05	6.6	2300	1672.0	467	236		348	121	1.47	0.51
	9	45	7.53	9.8	5.26	6.7	270	195.0	104	207		279	116	1.35	0.56
	10	30	5.68	5.0	3.70	7.0	200	170.0	104	237		261	85	1.10	0.36
	Average									233		320	111		

\* In these experiments, whole blood and urine were analyzed for urea-plus-ammonia nitrogen by the gasometric hypobromite method. The use of the hypobromite method in analyzing whole blood and urine has been demonstrated in our high-clearance patients to furnish whole blood clearance results not deviating by more than 10 per cent from simultaneous plasma clearance determinations in which plasma and urine were analyzed by the urease method. Hence, all clearance values have been tabulated as "plasma" clearances, and used as such in calculating ratios.

† Endogenous plasma creatinine too low to measure.

‡ Plasma creatinine level rising during this period.

§ Inulin 5 grams given intravenously during this period.

¶ Inulin 10 grams given intravenously during this period.

|| Creatinine 4 grams given by mouth during this period.

\*\* Creatinine 5 grams given by mouth during this period.

†† Casein hydrolysate 20 grams given intravenously during this period, with subsequent chill and rise of temperature to 103.4°. Clearance values in Periods 7 and 8 not used in calculating averages.

‡‡ To obtain "V," which is the urine flow per minute per 1.73 square meters of body surface, the observed urine flow has been multiplied by the "V factor," which is the ratio of 1.73 to the subject's surface area in square meters, determined by his age and height (15).

Casein hydrolysate,<sup>2</sup> prepared as a 10 per cent solution (6, 7) was given intravenously on at least one occasion to each of the low-clearance patients, and to S. G. in the high-clearance group. The amino acid mixture was given after 1 or 2 periods of simultaneous determination of inulin, urea, and creatinine clearances, and all clearances were again determined during 1 or 2 subsequent periods. The quantities of casein hydrolysate given to each patient are shown in Tables I and II.

## ANALYTICAL METHODS

Urea-plus-ammonia nitrogen in whole blood and urine was determined in some experiments by the hypobromite gasometric method of Van Slyke and Kugel (8); the experiments in which this method was used are indicated by an asterisk in Tables I and II. In the remainder plasma and urine urea nitrogen were determined by the gasometric urease method of Van Slyke (9).

<sup>2</sup>Furnished through the generosity of Mead Johnson and Co., Evansville, Indiana.

Creatinine was determined with the Summerson (10) photoelectric colorimeter by the method of Folin and Wu as modified by Miller and Winkler (11). The plasma values of the 2 control patients were corrected for non-creatinine chromogen by the specific enzymatic method of Miller and Dubos (12). In the high-clearance group the "endogenous" plasma chromogen levels were so low as to make accurate determination of non-creatinine chromogen impossible; indeed, in the case of R. M. there was no Jaffe reaction demonstrable in the plasma filtrate. In the single low-clearance patient (J. C.) in which it was determined, the non-creatinine chromogen was less than 10 per cent of the total chromogenic substance of the plasma; no corrections have been applied to the plasma values of the low-clearance group.

Plasma and urinary inulin were determined by the technique described by Alving, Rubin and Miller (13), which was modified slightly according to suggestions made by Dr. A. S. Alving in personal communications; the Summerson photoelectric colorimeter was employed.

TABLE II

*Nephrotic patients with low urea clearances. Comparison of inulin, creatinine, and urea clearances*

Subject	Period	Duration	Urine flow V <sub>1</sub> §§	Plasma levels			Urine levels			Clearances				Clearance ratios		
				Inu- lin	Creati- nine	Urea N	Inu- lin	Creati- nine	Urea N	Inulin	Endo- genous creat- inine	Exo- genous creat- inine	Urea	Endo- genous creat- inine: Inulin	Exo- genous creat- inine: Inulin	Urea: Inulin
A. C. ♀ 21 years (V factor§§=1.03) February 5, 1940	1	84	2.06		4.0	33.7*		34.2	203*	cc. plasma per minute	cc. plasma per minute	cc. plasma per minute	cc. plasma per minute			
	2§	60	2.65			32.7*		29.1	178*	17.6	19.7	14.0*	14.4*			
	3	60	3.57	30.5		34.4*	125	22.8	135*	14.7	21.0	14.0*	14.0*	1.43		0.95*
	4	60	3.04	25.5		33.6*	120	25.1	152*	14.3	19.2		13.7*	1.34		0.96*
	Average										14.5	19.4	13.6*			
Idem February 10, 1940	1	62	2.28		4.1	32.9*		33.0	188*		18.5		13.2*			
	2	60	3.69					24.1	114*		19.7		12.9*			
	3	57	3.92					21.2	111*		20.1		13.4*			
	4	60	1.88					34.3	203*		15.7		12.0*			
	5	62	3.30		8.1			32.3	131*				13.2*			
	6	64	3.24	37.7	14.2	31.8*	155	65.5	127*	13.5		14.9†	13.0*		1.10	0.96*
	7	59	2.65	31.2	16.2	31.5*	165	108.4	152*	14.1		17.7	12.9*		1.26	0.91*
	8††	58	3.84	28.2	15.8	32.5*	113	73.0	110*	15.5		17.7	13.1*		1.14	0.85*
	9	62	2.52	27.0	15.4	32.6*	128	94.9	155*	12.0		15.5	12.1*		1.29	1.01*
	Average										13.8	18.5	16.5	12.9*		
S. W. ♀ 4 years (V factor§§=2.95) February 13, 1940	1	57	2.28		2.7	44.6*		15.2	223*		12.8		11.4*			
	2	55	4.07					9.7	143*		14.7		13.0*			
	3	61	2.13					15.5	224*		12.3		10.7*			
	4†	62	1.90		4.3			35.0	238*				10.4*			
	5	68	2.17	44.1	10.4	43.5*	282	82.7	193*	13.9		17.2†	9.6*		1.24	0.69*
	6	54	2.41	39.8	13.5	42.6*	205	88.4	174*	12.4		15.7	9.9*		1.27	0.80*
	7††	62	2.09	35.8	12.8	46.0*	205	94.5	212*	12.0		15.4	9.6*		1.28	0.80*
	8	53	2.50	32.0	12.0	44.4*	140	76.8	193*	10.9		16.3	10.5*		1.50	0.96*
	Average										12.3	13.3	16.1	10.6*		

TABLE I

Nephrotic patients with high urea clearances. Comparison of inulin, creatinine, and urea clearances

Subject	Period	Duration	Urine flow V <sup>††</sup>	Plasma levels			Urine levels			Clearances				Clearance ratios	
				Inulin	Creatinine	Urea N	Inulin	Creatinine	Urea N	Inulin	Endogenous creatinine	Exogenous creatinine	Urea	Exogenous creatinine: Inulin	Urea: Inulin
		minutes	cc. per minute	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	cc. plasma per minute	cc. plasma per minute	cc. plasma per minute	cc. plasma per minute		
R. Q. ♀ 8 years (V factor <sup>††</sup> = 1.74) January 25, 1940	1	60	3.61			7.5*			230*				111*		
	2§	55	4.30			6.2*			177*				122*		
	3	60	6.68	11.6		6.2*	330		105*	190			113*		0.59*
	4	60	3.55	4.4		6.4*	280		192*	226			106*		0.47*
	Average										208		113		
Idem March 11, 1940	1	70	6.72		0.33	7.6		9.2	146		184		129		
	2	57	3.56					15.6	194		166		91		
	3	58	4.14					14.0	208		173		113		
	4¶	59	2.32		3.45	7.5		117.4	282				87		
	5	75	6.10		7.38	7.0		340.0	105			281	92		
	6	33	1.03	21.0	5.45	7.0	4300	2030.0	418	212		386	86	1.82	0.41
	7	32	2.40	14.6	4.55	6.5	1200	586.0	256	197		309	95	1.57	0.48
	8	41	3.84	10.4	3.78	6.5	450	220.0	128	166		223	75	1.34	0.45
	9	34	7.64	7.2	3.00	7.2	185	114.0	103	196		290	110	1.48	0.56
	Average										193	174	297	98	
S. G. ♂ 10 years (V factor <sup>††</sup> = 1.75) February 26, 1940	1	114	2.01		0.28	9.1		24.7	418		177		92		
	2	61	4.70					11.7	202		197		104		
	3¶	60	3.04		2.30			295.0	262			390†	88		
	4	60	6.25		3.67	8.9		216.0	123			368	86		
	5	60	1.81	18.1	2.26	9.1	1520	420.0	346	152		336	72	2.21	0.47
	6	60	1.20	8.7	1.32	9.4	1380	440.0	468	190		370	77	1.95	0.41
	7††	62	0.62	5.6	0.95	12.8	1160	403.0	316	129††		263††	28††	2.04	0.22
	8	58	1.69	3.2	0.75	14.4	620	123.0	478	327		276	61	0.84	0.19
	Average										171	187	366	86	
R. M. ♂ 3 years (V factor <sup>††</sup> = 2.75) April 16, 1940	1	55	2.99		†	7.1			323		†		136		
	2	60	11.10						76				119		
	3	58	6.55						116				103		
	4**	52	3.59		3.60			334.0	207			333†	105		
	5	50	7.05		11.96	6.7		634.0	101			373	105		
	6	35	10.80	51.5	11.12	6.7	1030	358.0	72	216		349	115	1.62	0.53
	7	30	6.96	20.0	9.40	6.6	760	400.0	95	265		296	100	1.12	0.38
	8	30	1.46	14.3	7.05	6.6	2300	1672.0	467	236		348	121	1.47	0.51
	9	45	7.53	9.8	5.26	6.7	270	195.0	104	207		279	116	1.35	0.56
	10	30	5.68	5.0	3.70	7.0	200	170.0	104	237		261	85	1.10	0.36
	Average										233		320	111	

\* In these experiments, whole blood and urine were analyzed for urea-plus-ammonia nitrogen by the gasometric hypobromite method. The use of the hypobromite method in analyzing whole blood and urine has been demonstrated in our high-clearance patients to furnish whole blood clearance results not deviating by more than 10 per cent from simultaneous plasma clearance determinations in which plasma and urine were analyzed by the urease method. Hence, all clearance values have been tabulated as "plasma" clearances, and used as such in calculating ratios.

† Endogenous plasma creatinine too low to measure.

‡ Plasma creatinine level rising during this period.

§ Inulin 5 grams given intravenously during this period.

|| Inulin 10 grams given intravenously during this period.

¶ Creatinine 4 grams given by mouth during this period.

\*\* Creatinine 5 grams given by mouth during this period.

†† Casein hydrolysate 20 grams given intravenously during this period, with subsequent chill and rise of temperature to 103.4°. Clearance values in Periods 7 and 8 not used in calculating averages.

‡‡ To obtain "V," which is the urine flow per minute per 1.73 square meters of body surface, the observed urine flow has been multiplied by the "V factor," which is the ratio of 1.73 to the subject's surface area in square meters, determined from his age and height (15).

Casein hydrolysate,<sup>2</sup> prepared as a 10 per cent solution (6, 7) was given intravenously on at least one occasion to each of the low-clearance patients, and to S. G. in the high-clearance group. The amino acid mixture was given after 1 or 2 periods of simultaneous determination of inulin, urea, and creatinine clearances, and all clearances were again determined during 1 or 2 subsequent periods. The quantities of casein hydrolysate given to each patient are shown in Tables I and II.

## ANALYTICAL METHODS

Urea-plus-ammonia nitrogen in whole blood and urine was determined in some experiments by the hypobromite gasometric method of Van Slyke and Kugel (8); the experiments in which this method was used are indicated by an asterisk in Tables I and II. In the remainder plasma and urine urea nitrogen were determined by the gasometric urease method of Van Slyke (9).

<sup>2</sup>Furnished through the generosity of Mead Johnson and Co., Evansville, Indiana.

Creatinine was determined with the Summerson (10) photoelectric colorimeter by the method of Folin and Wu as modified by Miller and Winkler (11). The plasma values of the 2 control patients were corrected for non-creatinine chromogen by the specific enzymatic method of Miller and Dubos (12). In the high-clearance group the "endogenous" plasma chromogen levels were so low as to make accurate determination of non-creatinine chromogen impossible; indeed, in the case of R. M. there was no Jaffe reaction demonstrable in the plasma filtrate. In the single low-clearance patient (J. C.) in which it was determined, the non-creatinine chromogen was less than 10 per cent of the total chromogenic substance of the plasma; no corrections have been applied to the plasma values of the low-clearance group.

Plasma and urinary inulin were determined by the technique described by Alving, Rubin and Miller (13), which was modified slightly according to suggestions made by Dr. A. S. Alving in personal communications; the Summerson photoelectric colorimeter was employed.

TABLE II

*Nephrotic patients with low urea clearances. Comparison of inulin, creatinine, and urea clearances*

Subject	Period	Duration	Urine flow V $\frac{1}{2}$	Plasma levels			Urine levels			Clearances				Clearance ratios		
				Inu- lin	Cre- ati- nine	Urea N	Inu- lin	Creat- inine	Urea N	Inulin	Endog- enous creat- inine	Exog- enous creat- inine	Urea	Endog- enous creat- inine: Inulin	Exog- enous creat- inine: Inulin	Urea: Inulin
A. C. ♀ 21 years (V factor $\frac{1}{2}$ = 1.03) February 5, 1940	1	84	2.06		4.0	33.7*		34.2	203*		17.6		12.3*			
	2 $\frac{1}{2}$	60	2.65			32.7*		29.1	178*		19.7		14.4*			
	3	60	3.57	30.5		34.4*	125	22.8	135*	14.7	21.0		14.0*	1.43		0.95*
	4	60	3.04	25.5		33.6*	120	25.1	152*	14.3	19.2		13.7*	1.34		0.96*
	Average										14.5	19.4	13.6*			
Idem February 10, 1940	1	62	2.28		4.1	32.9*		33.0	188*		18.5		13.2*			
	2	60	3.69					24.1	114*		19.7		12.9*			
	3	57	3.92					21.2	111*		20.1		13.4*			
	4	60	1.88					34.3	203*		15.7		12.0*			
	5	62	3.30		8.1			32.3	131*				13.2*			
	6	64	3.24	37.7	14.2	31.8*	155	65.5	127*	13.5		14.9†	13.0*		1.10	0.96*
	7	59	2.65	31.2	16.2	31.5*	165	108.4	152*	14.1		17.7	12.9*		1.26	0.91*
	8††	58	3.84	28.2	15.8	32.5*	113	73.0	110*	15.5		17.7	13.1*		1.14	0.85*
	9	62	2.52	27.0	15.4	32.6*	128	94.9	155*	12.0		15.5	12.1*		1.29	1.01*
	Average										13.8	18.5	16.5	12.9*		
S. W. ♀ 4 years (V factor $\frac{1}{2}$ = 2.95) February 13, 1940	1	57	2.28		2.7	44.6*		15.2	223*		12.8		11.4*			
	2	55	4.07					9.7	143*		14.7		13.0*			
	3	61	2.13					15.5	224*		12.3		10.7*			
	4†	62	1.90		4.3			35.0	238*				10.4*			
	5	68	2.17	44.1	10.4	43.5*	282	82.7	193*	13.9		17.2†	9.6*		1.24	0.69*
	6	54	2.41	39.8	13.5	42.6*	205	88.4	174*	12.4		15.7	9.9*		1.27	0.80*
	7††	62	2.09	35.8	12.8	46.0*	205	94.5	212*	12.0		15.4	9.6*		1.28	0.80*
	8	53	2.50	32.0	12.0	44.4*	140	76.8	193*	10.9		16.3	10.5*		1.50	0.96*
	Average										12.3	13.3	16.1	10.6*		



TABLE II (Continued)

Subject	Period	Duration	Urine flow $V$ §§	Plasma levels			Urine levels			Clearances				Clearance ratios		
				Inulin	Creatinine	Urea N	Inulin	Creatinine	Urea N	Inulin	Endogenous creatinine	Exogenous creatinine	Urea	Endogenous creatinine: Inulin	Exogenous creatinine: Inulin	Urea: Inulin
J. C. ♂ 7 years ( $V$ factor§§=2.26) January 29, 1940	1	104	2.77		2.0	38.1*		19.0	227*	cc. plasma per minute	cc. plasma per minute	cc. plasma per minute	cc. plasma per minute			
	2	47	4.23		1.9			12.5	136*		25.8		16.5*			
	3§	74	4.70			37.0*		11.2	130*		27.1		15.2*			
	4	60	4.74	63.3	2.0	37.2*	260	11.1	121*	19.6	27.1		16.5*	1.33		0.79*
	5	61	3.63	52.7		36.9*	240	12.9	149*	16.5	26.1		15.4*	1.41		0.88*
	Average									18.0	25.9		15.6*			
Idem February 1, 1940	1	101	3.14		2.0			15.4			24.2		14.4*			
	2	80	4.74			39.6*		8.9	120*		21.2		15.9*			
	3**	54	5.35		9.9			52.4	118*			28.2†	15.9*			
	4§	61	4.70		18.1	38.8*		107.2	126*			27.8†	15.2*			
	5	60	5.63	60.5	18.0	37.1*	200	96.0	115*	18.5	30.0		17.5*	1.62		0.95*
	6††	63	4.03	50.0	17.2	41.0*	220	116.0	162*	17.7	27.1		16.0*	1.53		0.90*
	7	57	5.05	44.4	16.7	41.9*	140	88.7	135*	16.0	26.9		16.3*	1.68		1.02*
Idem March 7, 1940	Average									17.4	22.7	28.0	15.9*			
	1	61	9.05		4.6	100.0		18.1	260		35.9		23.5			
	2	60	6.68					17.5	247		25.5		16.5			
	3	76	0.38					12.1	152				1.3			
	4¶	54	6.84		10.0			21.5	203				13.9			
	5§	66	3.62		17.1	98.6		51.3	182			10.9†	6.5			
	6	59	3.58	82.0	17.6		205	50.6	176	9.0	10.4		6.4	1.16		0.71
	7	70	3.60	74.0	16.6	92.5	170	46.9	178	8.3	10.2		6.9	1.23		0.83
	8††	140	2.99	60.0	16.0	96.4	195	55.9	222	9.7	10.5		6.9	1.08		0.71
	Average									—		—	—			

\* See asterisked footnote Table I.

† Plasma creatinine level rising during this period.

‡ Inulin 3.5 grams given intravenously and creatinine 1 gram given by mouth during this period.

§ Inulin 5 grams given intravenously during this period.

|| Inulin 5 grams given intravenously and creatinine 4 grams given by mouth during this period.

¶ Creatinine 2 grams given by mouth during this period.

\*\* Creatinine 5 grams given by mouth during this period.

†† Casein hydrolysate 5 grams given intravenously during this period.

‡‡ Casein hydrolysate 10 grams given intravenously during this period.

§§ See footnote ‡‡, Table I.

## CALCULATIONS

The urea, creatinine, and inulin clearances were calculated as the number of cc. of plasma cleared per minute per 1.73 square meters of surface area. The formula<sup>3</sup>

<sup>3</sup> The general clearance formula, introduced by Møller, McIntosh, and Van Slyke (14), is:

$$\text{Clearance} = \frac{U V}{P}$$

$U$  and  $P$  are, respectively, the concentrations in urine and plasma of the excreted substance—urea, creatinine, or inulin, etc.—and  $V$  is the urine flow expressed as cc. per minute per 1.73 square meters of body surface. The

of Møller, McIntosh and Van Slyke (14) was used. We have termed "endogenous" the creatinine clearances which were measured without administration of creatinine,

use of surface area in this calculation, as in the calculation of McIntosh, Møller and Van Slyke (15), makes the clearance formula give the same normal values for infants and children as for adults (15, 16). The surface area used in the calculation is estimated from the height and age of the child, as described by McIntosh *et al.* (15).

When the urine volume is above 2 cc. per minute per 1.73 square meters of body area, the urea clearance in man is independent of volume change; hence, urea clearances with  $V$  above 2 cc. have been termed "maximum"

TABLE III

Control subjects (recovered group). Comparison of inulin, creatinine, and urea clearances

Subject	Period	Duration	Urine flow V <sub>u</sub>	Plasma levels			Urine levels			Clearances				Clearance ratios	
				Inulin	Creatinine	Urea N	Inulin	Creatinine	Urea N	Inulin	Endogenous creatinine	Exogenous creatinine	Urea	Exogenous creatinine: Inulin	Urea: Inulin
H. G. ♀ 5 years ( $\bar{V}$ factor $\S$ = 2.10) April 22, 1940	1	60	1.47		0.48	14.6		49.6	1170		151		138		
	2	60	9.11					6.0	121		112		75		
	3	58	2.97					9.4	150		58		30		
	4†	60	3.25		0.70			18.1	322				72		
	5*	50	5.46		5.98	13.4		162.0	152				62		
	6	32	1.71	48.6	13.25	13.2	4160	1300.0	740	146		167	103	1.14	0.71
	7	30	3.42	28.1	11.12	12.5	1200	570.0	278	147		176	76	1.20	0.52
	8	31	13.50	18.8	8.92	12.4	157	122.0	78	113		185	78	1.64	0.69
	9	30	15.00	13.5	7.20	12.7	84	88.8	66	93		185	79	1.99	0.85
	10	30	5.04	9.5	6.14	12.5	165	165.0	144	87		135	68	1.55	0.78
	Average										117	107	170	78	
B. D. ♀ 6 years ( $\bar{V}$ factor $\S$ = 2.12) June 21, 1940	1	60	0.92		0.46	13.0		58.2	658		116		69		
	2	51	4.63					11.7	328		117		117		
	3†	123	0.67						451				40		
	4*	63	7.42		8.21	11.8		197.0	148				93		
	5	30	2.26	57.0	10.16	11.5	3870	626.0	339	154			67		0.43
	6	30	2.48	28.0	10.34	11.2	1725	737.0	324	152		176	72	1.16	0.47
	7	31	4.45	17.5	9.40	10.9	590	382.0	171	150		180	70	1.20	0.47
	8	29	4.09	12.5	8.38	9.9	450	387.0	186	147		189	77	1.29	0.52
	Average										151	116	182	76	

\* Inulin 10 grams given intravenously during this period.  
† Creatinine 4 grams given by mouth during this period.

‡ Creatinine 5 grams given by mouth during this period.  
§ See footnote ††, Table I.

and "exogenous" those which were determined after the blood creatinine content was increased by the feeding of creatinine. The values for the plasma concentrations of inulin and creatinine, in experiments where these substances were administered, were estimated for the mid-point of each period by interpolation on a graph, plasma concentration being plotted arithmetically against time. In each instance, the reported values for the inulin clearances were estimated from data obtained while the plasma level of inulin was falling; calculations of exogenous creatinine clearances were likewise made from data ob-

tained while the plasma creatinine concentration was falling, except where it is specifically stated otherwise in the tables. Since blood urea changed but little during the experiment, the plasma urea value directly obtained at the end of each period was used in calculating the urea clearance for that period.

## RESULTS

### Comparison of urea, inulin and creatinine clearances

clearances (14). When  $\bar{V}$  is between 0.5 and 2 cc., the urea clearance has been found to vary as the square root of the urine volume (14). Hence, for volumes within this range, the maximum urea clearance is calculated as

$$\frac{U}{P} \times \bar{V} \times \sqrt{\frac{2}{\bar{V}}} \text{ or } \frac{U}{P} \sqrt{2\bar{V}} \text{ in this paper. The clearances}$$

of inulin and creatinine are calculated simply as  $\frac{U}{P} \bar{V}$  for all urine volumes, since it was noted that a fall of  $\bar{V}$  to the range between 2 and 0.5 cc. did not decrease the clearance of inulin or creatinine.

The results are given in detail, with respect to each patient of the 3 groups, in Tables I, II and III. In general, all clearances were affected similarly in each group of patients. Those patients with a high urea clearance had elevated creatinine and inulin clearances. In the control group all three clearances were within the usual ranges of normal values (4, 15, 16, 17). In the low-clearance group all three clearances were depressed. The consistency of the results in each

group is best demonstrated by the ratio of urea clearance to inulin clearance, which is also shown in Tables I, II, and III. The use of the ratio for comparative purposes compensates in part for divergencies in calculated results due to incomplete voiding of urine.

J. C. (Table II) failed to excrete ingested water during the experiment of March 7. An abrupt drop in clearances occurred during the experiment. Soon after the close of Period 8 the patient manifested a generalized convulsion which we believe was a result of water retention.

#### *Administration of casein hydrolysate*

The intravenous administration of casein hydrolysate to low-clearance patients was not followed by a rise in the clearance values. When it was given to S. G. he developed a severe chill and hyperpyrexia, with the diverse effects on the clearances noted in Table I. He had tolerated similar injections without reactions during the preceding 6 months.

### DISCUSSION

#### *High clearance and glomerular filtration*

Our patients with high urea clearances showed similarly high inulin clearances. Therefore, if we assume with Chasis and Smith (18) that the inulin clearance is an accurate index of glomerular filtration, we may conclude that in our patients the rate of formation of glomerular filtrate was abnormally rapid.

The question remains, whether the doubling of the rate of glomerular filtration was due (1) to doubling of the renal blood flow, with a normal filtration fraction of about 20 per cent (4, 19); (2) to a doubling of the filtered fraction of the plasma water, with a concomitant rise in the "extraction percentage" (19) of inulin and urea; or (3) to a combination of both mechanisms. One might expect that the hypoproteinemia of nephrosis, with resultant decrease in plasma oncotic pressure, would induce glomerular filtration of an increased fraction of the plasma water. We have, however, been unable to find any consistent correlation between low plasma albumin and high urea clearance, since we have observed the high clearance to persist long after normal plasma protein concentration has been regained. It appears,

therefore, that some cause more dominant than hypoproteinemia is chiefly responsible.<sup>4</sup>

#### *Tubular excretion of creatinine*

We have estimated the relative output of creatinine by glomerular filtration and tubular excretion on the assumption that the inulin clearance measured glomerular filtration. From the data of each experiment the rate of total creatinine excretion and the rate of filtration of creatinine (inulin clearance  $\times$  plasma creatinine concentration) were calculated as mgm. per minute per 1.73 square meters of body surface and plotted for each period against the plasma creatinine concentration for that period. The periods both with and without creatinine feeding were included. With uniformity the curves obtained approximated straight lines, both for observed total creatinine excretion and for estimated glomerular filtration, at plasma levels up to 10 mgm. per 100 cc.

For numerical comparisons of the different subjects, excretions have been calculated for a constant plasma creatinine concentration of 10 mgm. per 100 cc., or 0.1 mgm. per cc. The calculations have been made as follows:

- (a) Total mgm. creatinine excreted per minute = (cc. plasma cleared of creatinine per minute)  $\times$  0.1
- (b) Mgm. creatinine filtered by glomeruli per minute = (cc. plasma cleared of inulin per minute)  $\times$  0.1
- (c) Mgm. creatinine excreted by tubules per minute =  $a - b$ .

The values for plasma creatinine clearance used in formula *a* and inulin clearance used in formula *b* are the means of the determinations in each subject.

<sup>4</sup> There is one type of control that our data lack, *viz.*, the estimation of urea clearances on entirely normal children placed on the same régime of diet (salt poor, 3 grams of protein per kilo) and activity as our patients. The possibility that under these conditions normal children might show higher than ordinary clearances is not excluded by our data, nor by any that we have found in the literature. The absence of such high values in normal children on unrestricted diets (16) makes their occurrence seem improbable, but one cannot say that it is absolutely excluded.

The results of the calculations are given in Table IV. They indicate that the estimated tubular excretion of creatinine in the 3 groups parallels glomerular filtration, the mean tubular excretion of the high-clearance group being 12.9 mgm. per minute and that of the low-clearance group 0.6 mgm., compared with 4.2 mgm. for the controls.

TABLE IV

*Rate of excretion of creatinine by all subjects estimated for a plasma creatinine concentration of 10 mgm. per 100 cc., corrected to a body surface area of 1.73 square meters*

Patient	Total	Filtered	Secreted
	mgm. per minute	mgm. per minute	mgm. per minute
<i>Recovered group</i>			
H. G.	17.0	11.7	5.3
B. D.	18.2	15.1	3.1
Average	17.6	13.4	4.2
<i>High-clearance group</i>			
R. Q. March 11, 1940	29.7	19.3	10.4
S. G.	36.6	17.1	19.5
R. M.	32.0	23.3	8.7
Average	32.8	19.9	12.9
<i>Low-clearance group</i>			
J. C. February 1, 1940	2.80	1.74	1.06
March 7, 1940	1.05*	0.90*	0.15*
S. W.	1.61	1.23	0.38
A. C.	1.65	1.38	0.27
Average	2.02	1.45	0.57

\* Not included in average—see text.

### CONCLUSIONS

The persistent, abnormally high urea clearance, as great as 150 to 200 per cent of normal average, observed in certain children with the nephrotic syndrome, is a manifestation of generally increased renal excretory activity, since the inulin and creatinine clearances are also elevated above normal to approximately the same degree.

Insofar as tubular activity can be estimated from the ratio of exogenous creatinine clearance to inulin clearance, it appears that this activity is as much accelerated as is glomerular filtration.

Intravenous injection of amino acids did not increase the clearances in patients with diminished renal function.

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# SPONTANEOUS RECOVERY FROM NUTRITIONAL MACROCYTIC ANEMIA IN YOUNG SWINE FOLLOWING INITIAL ESTRUS

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Spontaneous recovery from a severe macrocytic anemia produced experimentally in young swine by a prolonged partial deficiency of the vitamin B-complex has not been reported previously. The present communication deals with such a study.

or combination of synthetic factors has proven effective as the extrinsic factor.

Due to the varied hematopoietic response of different species to the same treatment, any study involving anemia demands care in the selection of

TABLE I  
Correlation of age, sex, weight, diet and performance of all the animals

Swine number	Group	Sex	Initial age	Weight			Diet	Time* until severest anemia	Time* until cure of anemia†	Type of anemia	Duration of experiment	Fate of animal
				Initial	Anemia	Final						
			weeks	lbs.	lbs.	lbs.		weeks	weeks		weeks	
1	I	M	14	46	52	52	A	22		Microcytic Microcytic	22	D
2	I	M	13	33	25	25	A	8			8	D
3	I-A	F	21	85		261	A				17	S
4	I-A	F	21	98		268	A				17	S
5	I-A	F	21	92		242	A				17	S
6	II	F	14	45		30	B				5	D
7	II	F	13	50		30	B				7	D
8	III	F	14	37	193	369	D	18	40	Macrocytic Macrocytic	76	S
9	III	F	13	24	143	254	D	18	40		76	S
10	IV	F	14	40		29	E				8	D
11	IV	F	13	36		30	E				8	D
12	V	F	14	45	115	254	F	18	45	Macrocytic Macrocytic	76	S
13	V	F	13	34	58	341	F	15	28		76	S
14	VI	F	16	60	94	339	G	19	21	Macrocytic	55	D
15	VI	F	21	55		52	G				3	D
16	VII	F	9	36		215	C				22	D

D = died. S = sacrificed.

\* This indicates the time from the beginning of the experiment.

† For determining this point, we arbitrarily established 6 million red cells (approximately average initial value) as a return to normal. Scarborough (21) gives 6.74 million as the average normal red blood count for the pig, and 5 to 9 million as the normal range. Values finally reached by all pigs with macrocytic anemia were: red blood cells 7.5 to 8 million.

Anemias associated with a deficiency of factors of the vitamin B-complex have been reported from many different laboratories (1 to 16) as occurring in different species of animals. Castle's extrinsic factor required for the treatment of pernicious anemia in man is found in such natural sources of the B-complex as yeast, beef, wheat germ, rice polish, etc. But, as yet, no single synthetic factor

an experimental animal. The pig, which stores the pernicious anemia curative factor in its liver, was selected for this study because its hematopoietic response is more nearly like that of the human. Sixteen animals were used in the experiment (see Table I for age, weight, sex, diet, grouping, etc.). The long experimental period, 76 weeks in the case of the pigs with macrocytic

anemia, served to bring out certain points not previously noted.

### EXPERIMENTAL

Pure-bred Duroc Jersey pigs farrowed at the North Carolina State College Experimental Farm, Raleigh, N. C., were used in this study. They were kept in individual cement-floored, inside pens, each with its own outside run. Wood shavings served as bedding. The animals were fed twice daily and the food consumption was recorded. Hematologic studies were made weekly on a typical animal from each group until significant changes were observed, then all the animals were studied weekly.

quantitative measurements and for making fixed smears. Hemoglobin was measured by a Sahli hemoglobinometer which had been standardized. Reticulocyte counts were made on wet preparations stained with brilliant cresyl blue. Mean corpuscular volume and mean corpuscular hemoglobin content were determined by Wintrobe's method (16a).

*Diets.* The chief problem was one of selecting a diet deficient enough to produce anemia, and yet sufficiently adequate to permit time for its development. A too drastically deficient diet results in death before marked signs or symptoms develop. Both natural and synthetic diets were employed.

TABLE II  
Blood changes

Swine number	Group	Red blood cells millions			Hemoglobin grams per cent			Mean corpuscular volume cubic micra			Mean corpuscular hemoglobin content grams $\times 10^{-12}$			White blood cells		
		Initial	Anemia	Final	Initial	Anemia	Final	Initial	Anemia	Final	Initial	Anemia	Final	Initial	Anemia	Final
1	I	6.51	1.85	1.85	12.2	3.4	3.4	64	48	48	19	18	18	14,843	29,950	29,950
2	I		5.96	5.96		8.4	8.4		49	49		14	14		15,000	15,000
3	I-A	7.07		9.0	12.0		18.0	53		57	17		20	17,950		14,150
4	I-A	7.52		7.73	14.0		16.6	60		61	19		21	14,550		14,000
5	I-A	6.99		9.07	12.2		18.0	46		57	17		20	24,900		14,550
6	II	5.77		6.17	10.9		11.2	66		62	19		18	20,000		17,100
8	III	6.48	2.96	8.21	12.1	7.4	15.0	61	71	57	19	25	18	15,500	20,150	14,150
9	III		2.72	7.92		7.6	14.8		97	61		28	19		19,250	15,000
10	IV	5.29		6.04	10.4		11.2	70		55	19		19	17,000		20,000
12	V	5.07	3.93	7.55	10.0	9.0	13.0	69	81	60	20	23	17	14,900	18,000	20,900
13	V		2.76	7.95		5.0	14.3		76	54		24	18		24,800	14,500
14	VI	8.68	5.54	8.57	14.0	14.0	16.2	51	74	65	16	26	19	19,000	23,000	19,800
16	VII	7.61		9.0	13.6		15.4	60		56	18		17	16,500		26,000

Gastric acidity and stool fat determinations were made periodically. Necropsies were performed on most of the pigs. The findings, however, were not significant as far as the deficiency state was concerned since all the deficient animals living longer than 8 weeks had returned to normal by the end of the experiment.

The weanling pigs, having an average initial weight of 39 lbs., used in Groups I to V, inclusive, were paired litter mates. Three heavier animals with average initial weight of 92 lbs. were used in Group I, but because of their very different behavior were designated as Group I-A. The one surviving pig of Group VI was somewhat older and had an initial weight of 60 lbs. Pig 16, a young weanling of 36 lbs., and thus comparable to those of the first five groups, served as a positive control subsisting on a diet containing brewer's yeast at a level of 10 per cent for 22 weeks.

*Blood studies.* Blood from the jugular vein was collected in tubes containing potassium oxalate (2 mgm. per cc. of blood drawn). Venous blood was used for all the

### Natural diet

The pigs in Group I were given the following modification of Goldberger's blacktongue-producing diet number 123 of natural foodstuffs (17).

### Diet A

	per cent
Corn meal.....	63.0
Blackeyed peas.....	8.0
Casein.....	9.0
Cane sugar.....	5.0
Rice polish.....	6.0
Cotton seed oil.....	5.0
Cod liver oil.....	2.0
Sodium chloride.....	0.5
Calcium carbonate.....	1.5

This diet is almost identical with one used by Miller and Rhoads (4) for the production in swine of a mild anemia which was microcytic in some individuals and macrocytic in others.

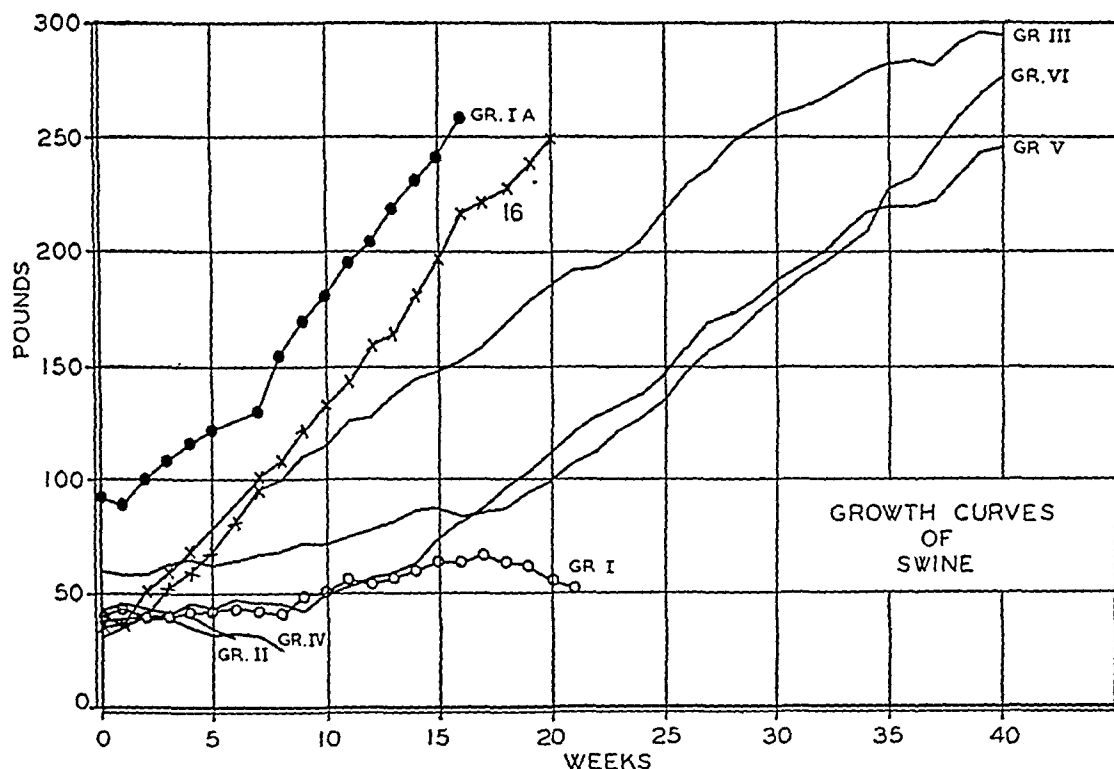


FIG. 1. GROWTH CURVES OF SWINE ON THE FOLLOWING DIETS:

Group I, weanling animals on the blacktongue-producing diet; Group I-A, older animals on the same diet; Group II, basal diet (synthetic vitamin B-complex free diet) + vitamin B<sub>1</sub>; Group III, basal diet + vitamin B<sub>1</sub> + yeast (4 per cent); Group IV, basal diet + vitamin B<sub>1</sub> + nicotinic acid + riboflavin; Group V, basal diet + vitamin B<sub>1</sub> + rice polish concentrate + yeast (2 per cent); Group VI, basal diet + vitamin B<sub>1</sub> + yeast (3 per cent); Pig 16 (positive control), basal diet + yeast (10 per cent).

Among the 5 pigs in Groups I and I-A receiving the diet, results appeared to depend largely on their initial weight. Both of the 2 younger pigs (1 and 2) developed microcytic anemia, one mild, and the other very severe. The 3 older pigs (3, 4 and 5) failed to develop anemia. The additional weight with the accompanying vitamin storage appeared to protect them against the deficiencies encountered. Further data concerning these animals will be found in Tables I and II and Figure 1.

Gastric analyses indicated free hydrochloric acid in all 5 pigs throughout the experiment.

At necropsy pig 2 showed enlarged and infected lymph nodes at the base of the neck, and small ulcers profusely distributed throughout the large intestine.

#### Synthetic diets

The basal diet which served as the foundation for all of the synthetic rations in these experi-

ments is essentially vitamin B-complex-free. Its composition follows:

	per cent
Casein .....	18
Cane sugar .....	67
Cotton seed oil .....	10
Cod liver oil .....	2
Salts <sup>1</sup> .....	3

This diet, when supplemented with an adequate source of the vitamin B-complex (brewer's yeast at a level of 10 per cent), was readily consumed,

#### <sup>1</sup> Salt mixture:

	per cent
Bone meal .....	57.8
Sodium chloride .....	24.4
Limestone .....	12.2
Iron sulfate .....	3.7
Magnesium oxide .....	1.2
Copper sulfate .....	0.3
Manganese sulfate .....	0.1
Zinc oxide .....	0.1
Cobalt carbonate .....	0.1
Potassium iodide .....	0.1



and resulted in good growth and freedom from anemia in spite of the fact that no provision was made for supplying vitamin C.

To reduce the degree of vitamin B-complex deficiency, supplements of the various factors of the vitamin B-complex in both synthetic and natural form were added to the basal diet as follows:

Group	II	IV	V	VI	III	VII
Diet.....	B	E	F	G	D	C

Daily supplements to the basal diet

	mgm.	mgm.				
Thiamin <sup>2</sup>	2.5	2.5	2.5 mgm.	2.5 mgm.	2.5 mgm.	
Nicotinic acid		25.0				
Riboflavin		0.5				
Rice polish concentrate <sup>3</sup>			30.0 cc.			
Brewer's yeast			2%	2%	4%	10%

Neither of the diets B or E, having only synthetic vitamin supplements, proved adequate. All pigs on these diets died before anemia developed (Groups II and IV, Table I).

However, natural sources of the vitamin B-complex, such as yeast and rice polish concentrate, when fed at the proper level, resulted in a partial prolonged deficiency which served our purpose in producing a macrocytic anemia. It was necessary to adjust the levels of these supplements slightly as the experiment proceeded.

*Diets on which macrocytic anemia was produced*

(a) *Diet F (basal diet supplemented with rice polish concentrate and brewer's yeast at a 2 per cent level; Group V, Pigs 12 and 13).* At the beginning of the experiment no yeast was incorporated in the basal diet and only 15 cc. of rice polish concentrate were given. At the end of 8 weeks the daily dose of rice polish concentrate was increased from 15 to 30 cc. This resulted in some general improvement but it was insufficient to insure time for the development of anemia. Since the response was not commensurate with the increase in active supplement, the effect was augmented by introducing brewer's yeast into the basal diet at a level of 2 per cent. With these alterations the pigs gained weight and, during a

period of 15 to 23 weeks, gradually developed a macrocytic anemia (Figure 1, Group V; Figure 3, D and E). Both animals had an occasional steatorrhea. Pig 13 developed achlorhydria, but Pig 12 retained its gastric acidity throughout the experiment. Both pigs developed stiff legs. The blood changes are recorded in Table II.

(b) *Diet G (basal diet with brewer's yeast incorporated at a level of 2 to 3 per cent replacing an equivalent amount of sugar; Group VI, Pigs 14 and 15).*<sup>4</sup> Exact comparisons cannot be made between the different levels of yeast since Pig 14, the surviving pig on this diet, was older and considerably heavier than the pigs on the 4 per cent and 10 per cent levels. The importance of this will be discussed in more detail later because of its bearing on the expected results. Pig 14, a healthy young pig with an initial weight of 60 lbs., was given the basal diet alone for 1 week. Yeast was then incorporated into the basal diet at a level of 2 per cent for 15 weeks, when the pig began to vomit and lose weight. It was obvious that the pig could not survive long on this regime. Consequently, the yeast level was raised to 4 per cent for 2 weeks, then reduced to 3 per cent for 19 weeks, to 2 per cent for 11 weeks and to 1 per cent for 3 weeks. Yeast was then completely removed from the diet and the pig died 3 weeks later. The blood changes are recorded in Table II.

*Neurologic changes.* At the end of 18 weeks this pig had developed stiff legs resulting in a very awkward walk with a tendency to lift the feet rather high. Increased excitability was noted in the animal at this time. The pig seemed to grow gradually more irritable and tense when, during the thirty-first week, it had an epileptiform seizure. This was never repeated, nor were any other neurologic signs noted, but the animal continued to be somewhat more excitable than normal. Shortly before death there was a generalized hemorrhagic diathesis characterized by nose bleed and hemorrhage into the skin. There was no vitamin C in the diet, and since these symptoms were suggestive of scurvy, large doses of ascorbic acid

<sup>2</sup> The thiamin used in this study was kindly supplied by Merck and Company, Rahway, New Jersey.

<sup>3</sup> A part of the rice polish concentrate (chicken riboflavin test supplement) used was kindly supplied by the Vitab Company, Emeryville, California.

<sup>4</sup> Pig 15 was obviously not in normal condition at the start of the experiment. It was 21 weeks old and weighed 55 lbs., as compared with 92 lbs., the average weight of other pigs of that age. It developed extreme weakness of the hind legs in 2½ weeks, became completely paralyzed and died at the end of 3 weeks.

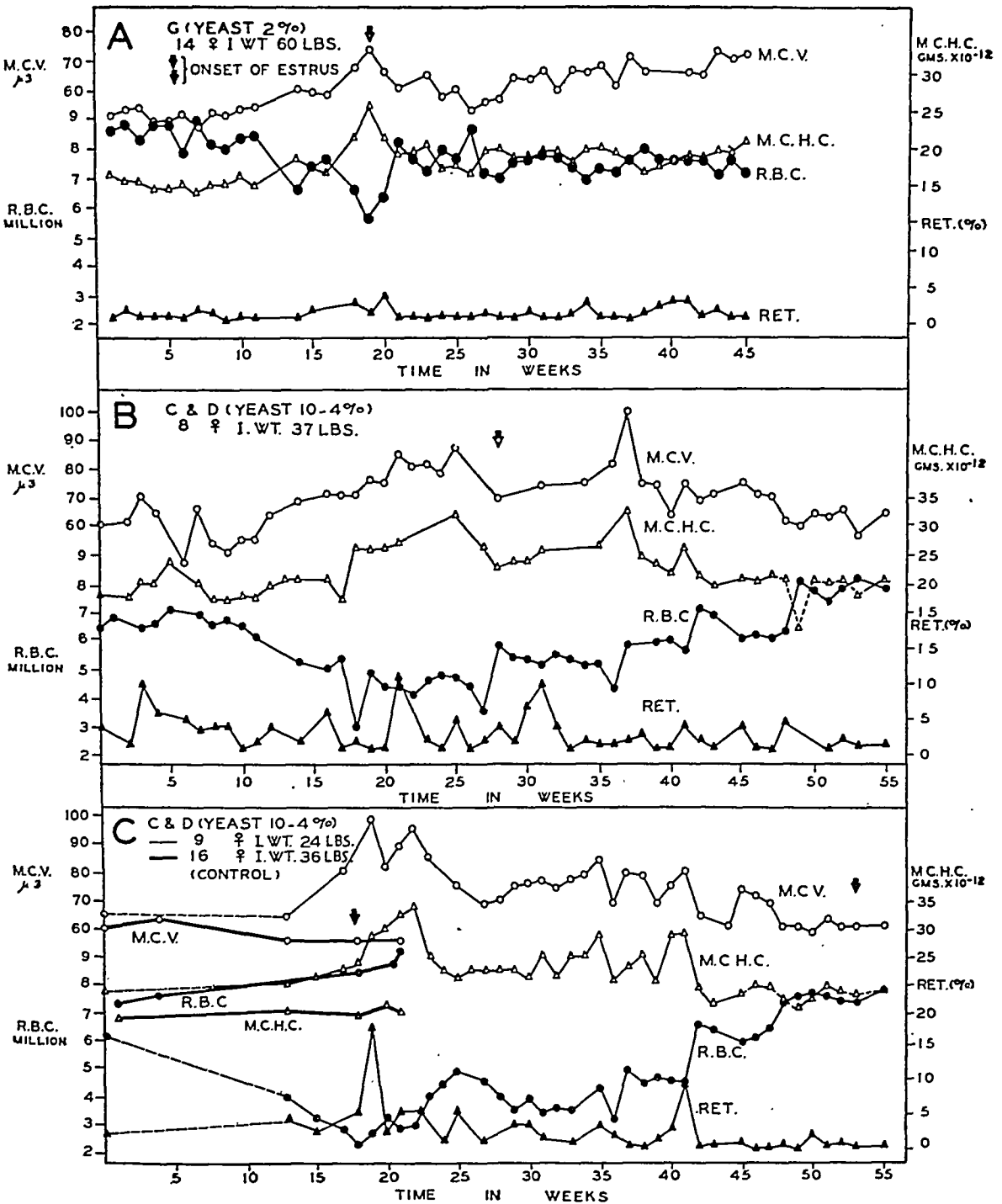


FIG. 2. CHARTS OF THE RED BLOOD COUNTS, RETICULOCYTES, MEAN CORPUSCULAR HEMOGLOBIN CONTENT AND MEAN CORPUSCULAR VOLUME OF:

A. Fig. 14, an animal slightly heavier than the others, receiving a synthetic diet with yeast at a level of 3 per cent; B. Fig 8, a lighter animal receiving a similar synthetic diet with yeast at a level of 4 per cent; C. Fig 9, a light animal comparable to Fig 8. The hematology chart of Fig 16, positive control receiving yeast at level of 10 per cent, is superimposed for comparison. At this level there is a gradual steady increase in the red cell count, whereas a similar weanling on a 4 per cent level shows a gradual decrease in the red cell count with a subsequent return to normal.

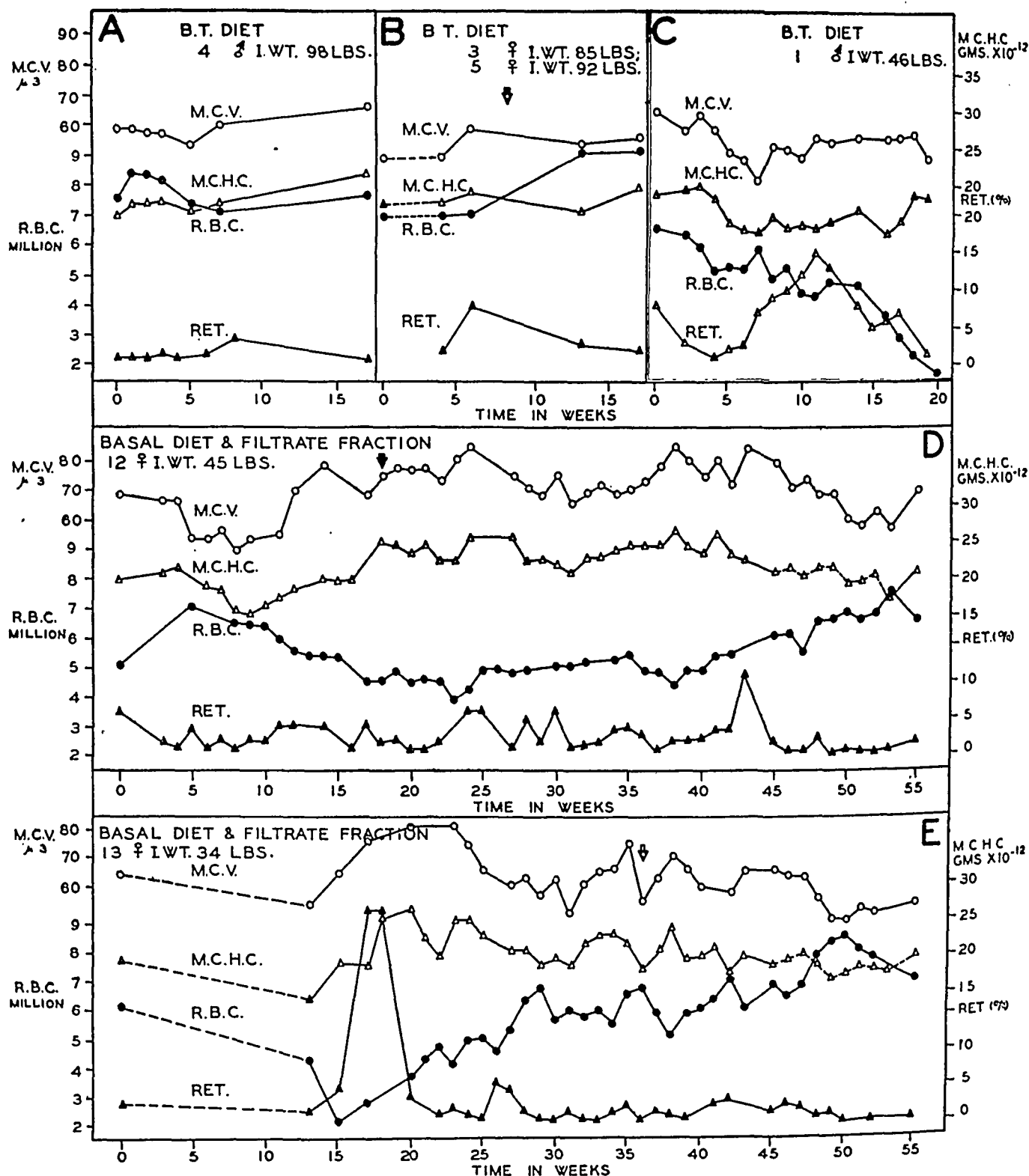


FIG. 3. CHARTS OF THE RED BLOOD COUNT, RETICULOCYTES, MEAN CORPUSCULAR HEMOGLOBIN CONTENT AND MEAN CORPUSCULAR VOLUME OF:

A. Pig 4, a slightly heavier male receiving the natural blacktongue-producing diet; B. average values for 2 comparable females (the 3 pigs having an initial weight of 92 lbs.) receiving the same diet; C. Pig 1 having an initial weight of 46 lbs. on the same blacktongue-producing diet and showing the development of a severe microcytic anemia; D. and E. Pigs 12 and 13, weanlings with initial weights of 45 and 34 lbs., receiving the synthetic basal diet + vitamin B<sub>1</sub> + rice polish concentrate + yeast (2 per cent), and showing the development of a macrocytic anemia with subsequent spontaneous recovery.

were given by mouth and intraperitoneally but without effect. Death due to pneumonia was demonstrated at autopsy.

(c) *Diet D (basal diet with yeast incorporated at a level of 4 per cent; Group III, Pigs 8 and 9).* These animals consumed a diet containing yeast at a level of 10 per cent (Diet C) for 8 weeks. During this time the pigs appeared normal in every way. The yeast was then decreased from 10 per cent to 4 per cent. Although both pigs continued to grow on this regime at an approximately normal rate, they developed a very severe macrocytic anemia during the subsequent 10 weeks. The blood changes are recorded in Table II.

Pig 9 developed gastric achlorhydria after about a year on the diet but Pig 8 continued to show free gastric hydrochloric acid throughout the experiment.

#### *Positive control*

(d) *Diet C (basal diet with yeast incorporated at a level of 10 per cent; Group VII, Pig 16).* Only one animal consumed Diet C for a sufficiently long experimental period to serve as a positive control. Yeast at this level completely protected this pig against the development of anemia. Growth was excellent (Figure 1), the animal remained free from anemia throughout the experiment (Figure 2, C), and initial estrus occurred at the expected time. Blood changes are recorded in Table II.

#### DISCUSSION

Four factors affect the results obtained in any nutrition experiment: (1) the degree of deficiency of the diet; (2) the vitamin storage of the animal at the beginning of the experiment; (3) any coincident physiologic stress; (4) the presence of infection. The first is usually determined arbitrarily by the investigator, the second by the age and previous diet of the animal, the third chiefly by age, and the fourth is purely a matter of chance unless infection is induced as a part of the experiment.

In the experiments reported here, the young pigs of Groups III, V, and VI developed a macrocytic anemia which was most acute during and shortly after the period which corresponds roughly

to adolescence in the human. This anemia cleared up gradually after time had elapsed for the establishment of normal estrous rhythm and while the animals were still consuming essentially the same diet. Initial estrus occurs in the pigs of the North Carolina State College Experimental Farm at from 20 to 35 weeks of age. Examination of Figure 2, A, B and C and Figure 3, D and E, indicates that the low point of the anemia was reached after 15 to 22 weeks on the experiment when the pigs were between 28 and 35 weeks old.

TABLE III  
*Relationship of body weight and diet to onset of estrus and development of anemia*

Pig	Initial weight	First estrus	Diet	Macrocytic anemia
	lbs.	weeks on experiment		
16 (control)	36	18	Adequate	
14	60	19	Inadequate	+
12	45	18	Inadequate	++
8	37	28	Inadequate	+++
13	34	36	Inadequate	+++
9	24	53	Inadequate	++++

Pig 14 had additional estrous periods at 23 and 50 weeks; Pig 12 at 53, 61, 67 and 73 weeks; Pig 8 at 36, 40, 58, 61, 73 and 77 weeks and, after being placed back in stock, gave birth to a litter of 6 live pigs; Pig 13 at 53 and 74 weeks, and Pig 9 one at 65 weeks. The anemia began to improve gradually after the initial estrus except in Pig 9 where improvement preceded this long delayed initial estrus by a few weeks.

Much evidence indicates that the factors of the vitamin B-complex play a part in maintaining the gonads as well as the hematopoietic system. Concrete evidence of the effect of vitamin B-complex deficiency on the estrous cycle of rats has been offered by Richter and Hawkes (18) in their carefully controlled experiments. These investigators noted that, if from an otherwise adequate diet, yeast, serving as the sole source of the vitamin B-complex, was withdrawn, it resulted in loss of estrus in 14 days and marked atrophy of the ovaries and uterus in 40 days.

If we may assume, then, that the comparatively low level of the vitamin B-complex contained in the diets described above must be shared both by the blood-forming elements and the sex endocrine system, any heightened activity of the latter system will increase its requirement of the vitamin B factors and thus precipitate a more marked deficiency which will be reflected in the former sys-

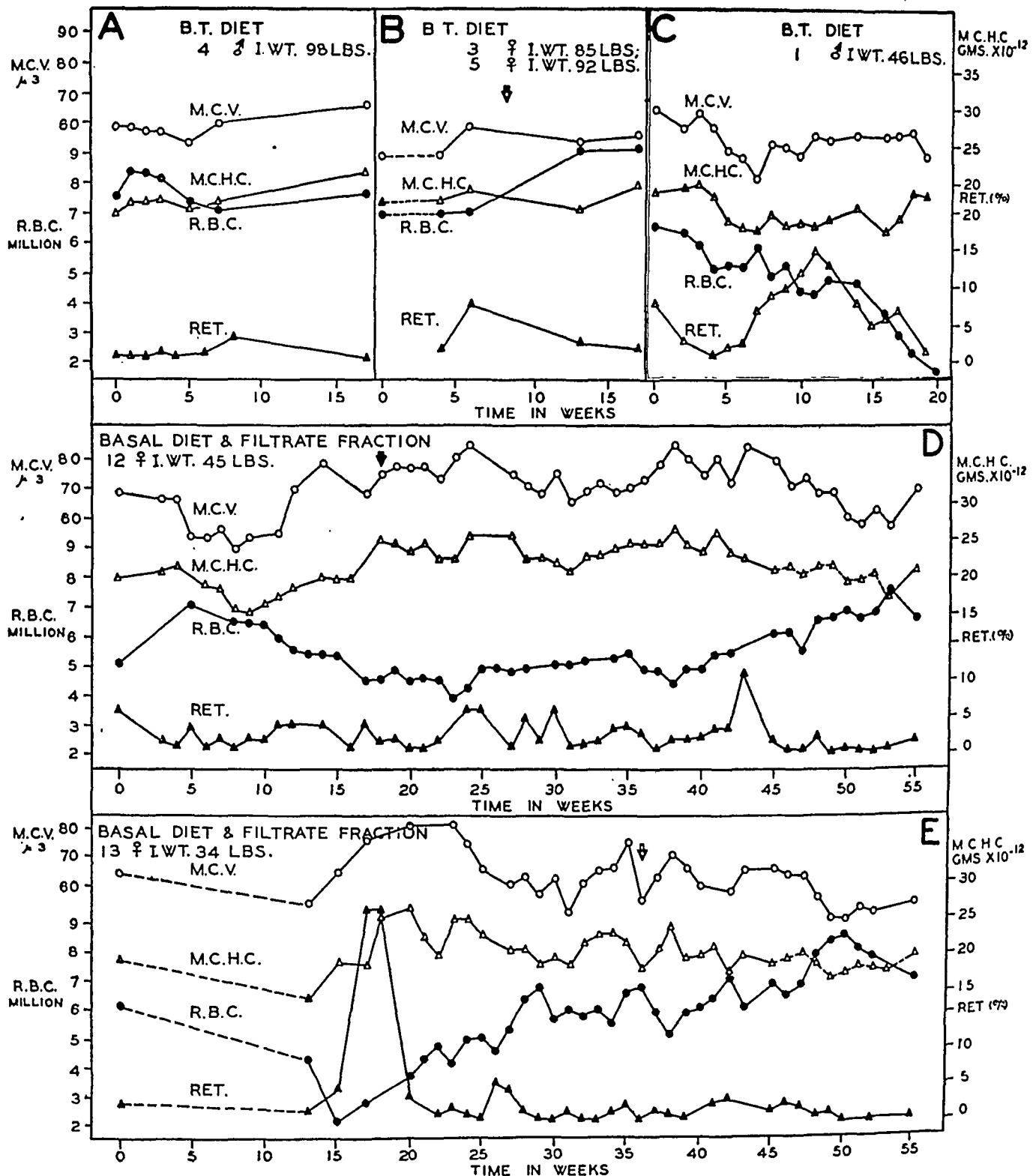


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tem. One would expect equilibrium to be reestablished only after the strain of the onset of estrus had passed and sexual stability had been established. This is just what occurred. Not only did 5 pigs of Groups III, V and VI develop a macrocytic anemia, but there was a definite postponement of estrus in the 3 pigs having the most severe anemia, namely, Pigs 8, 9 and 13. Onset of estrus was noted in Pigs 12 and 14 at the expected time. Both these animals were heavier, having an initial weight of 45 and 60 lbs., respectively, as compared with the 37, 24 and 34 lbs. of the other 3. This additional weight with the increased vitamin storage could easily account for the greater protection afforded both systems, namely, a much milder anemia and no demonstrable interference with the onset of estrus.

We believe that the macrocytic anemia observed in these animals is the most severe yet produced experimentally in pigs. Undoubtedly, the prolonged *partial* deficiency of the vitamin B-complex in the diet, the unstable condition of the animals due to their initial age and weight, and the long duration of the experiment all contributed to this result. As one would expect in any macrocytosis, the mean corpuscular volume and mean corpuscular hemoglobin content increased proportionally as the red count fell (Figure 2, A, B and C; Figure 3, D and E). The mean corpuscular volume rose to a maximum of 97 cubic micra in Group III and 81 cubic micra in Group V, the maximum mean corpuscular hemoglobin content being  $28 \times 10^{-12}$  grams and  $24 \times 10^{-12}$  grams, respectively.

Two other groups of investigators have produced macrocytic anemia experimentally in swine; Miller and Rhoads in 1935 (4) and Wintrobe and his coworkers in 1939 (16b). The former group produced anemia on a diet quite similar to our Diet A. This resulted in microcytic anemia in some individuals and macrocytic in others. The highest mean corpuscular volume reported was 62 cubic micra. Compared with ours, this is low but it represents a significant rise over the initial value of the individual pig. Wintrobe and his associates produced some degree of macrocytosis on synthetic diets similar to ours in which reduced levels of yeast supplied the factors of the vitamin B-complex. The highest mean corpuscular vol-

ume reported by these investigators was 68 cubic micra.

The blood smear of Pig 9 of our series showed large numbers of reticulocytes but there was no reason to suspect a hemolytic process at this time. There was never any clinical evidence of blood loss or of jaundice, and the giant size of the majority of the reticulocytes makes the presence of a hemolytic process improbable. Pigs which developed macrocytic anemia in similar experiments performed by Wintrobe showed no change in their icteric indices (16a).

All of the mineral elements thought to be concerned in any way with hemoglobin formation, either directly or catalytically, such as iron, copper, manganese, cobalt, etc., were supplied adequately in the salt mixture of the synthetic diets. Therefore, we feel that the anemia produced on the synthetic diets was in no way referable to a mineral deficiency.

Chick and her collaborators (7) found a level of 4 per cent yeast adequate in supplying all the B vitamins required by young pigs (initial weight 51 to 70 lbs.) during an experimental period of 9 weeks. Our experience with pigs having an initial weight of 24 to 37 lbs. observed over a 76-week experimental period indicates that, although approximately normal growth ensues, this level of yeast does protect against the development of macrocytic anemia. Our positive control pig tested for a period of 22 weeks showed excellent growth, well-being, and freedom from anemia, and exhibited an improvement in the blood during this period, indicating that a level of 10 per cent brewer's yeast was adequate to maintain the blood-forming elements in this pig. Since levels between 4 per cent and 10 per cent were not tried, we do not know the minimum protective level for a pig of this weight (35 to 40 lbs.).

Smith and Otis (19) have presented evidence that female rats use low levels of iron in the diet more efficiently for hemoglobin building than do males. In this connection it is interesting that, of the 3 animals in Group I-A, the one male increased his hemoglobin from 14 to 16 grams during the experimental period, while the 2 females increased theirs from 12 to 18 grams (average values) during the same period. The number of

animals used, however, is much too small to draw conclusions.

All the neurologic symptoms noted in our animals have been observed previously by others (4, 7, 16b). The occurrence of one epileptiform seizure in Pig 14 parallels the experience of Chick and her collaborators who observed sporadic seizures in pigs on a borderline level of vitamin B<sub>6</sub>. Where the B<sub>6</sub> deficiency was more acute, the seizures increased in number and intensity until the eluate fraction supplying the B<sub>6</sub> was administered. A diet in which yeast at a level of 2 to 3 per cent supplies the B-factors results in a borderline level of vitamin B<sub>6</sub> and explains the occurrence of only one such seizure in our animal.

#### SUMMARY AND CONCLUSIONS

A severe macrocytic anemia was produced in weanling pigs on a prolonged partial deficiency of the vitamin B-complex.

Brewer's yeast at a level of 4 per cent was not adequate under the conditions of these experiments to protect weanling pigs (weighing 45 lbs. or less) from developing macrocytic anemia. A 10 per cent level of yeast, however, was quite satisfactory for this purpose in the one animal tested for 22 weeks.

Spontaneous cure of the anemia ensued while the pigs remained on the same diet and without treatment.

A possible explanation for the development of anemia on a diet which later is adequate for its cure is offered in the physiological stress and strain preceding sexual maturity, which apparently corrects itself after sexual stability is accomplished.

#### ACKNOWLEDGMENTS

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# STUDIES ON THE CHEMICAL DIAGNOSIS OF PELLAGRA (NICOTINIC ACID DEFICIENCY) <sup>1</sup>

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Since the demonstration that nicotinamide is the pellagra-preventing factor (1), attempts have been made to analyze this substance in blood and urine as a measure of nicotinic acid nutrition. Chemical methods and biological methods, to be considered subsequently, have been used. The chemical methods have been based, most commonly, upon the development of colored products by the reaction of various pyridine compounds with cyanogen bromide and aromatic amines, as described by König (2).

This reaction is not specific for nicotinic acid. Other pyridine compounds may give similar colored products. Elvehjem (3) has found that, in the analysis of animal tissues, the reaction as we have applied it gave results quite comparable to those which he obtained by animal assay. He found that, in vegetable materials, the chemical method gave too high values, presumably due to other pyridine compounds. Consequently, blood analyses might be expected to avoid the possible errors due to the concentration of those other compounds in the urine.

Nicotinic acid does not occur, as such, in appreciable quantities in the blood or urine except shortly after the taking of large doses of it (4, 5). In the blood, it occurs chiefly in the corpuscles as a constituent of coenzymes I and II.

Nicotinic acid in the blood has been determined by four different procedures: chemical estimation using the cyanogen bromide and aromatic amine reagents, measurement of coenzymes I and II as the essential growth factor for *H. influenzae* or *H. parainfluenzae*, estimation of total nicotinamide

(both free and in the coenzymes) as the growth factor for *B. proteus*, and measurement of coenzyme I by a specific fermentation method. The results obtained with both normal and deficient subjects are listed in Table I.

TABLE I  
Reported values for nicotinic acid content of the  
blood of human subjects

Author (Reference)	Method	Whole blood values*	
		Normal	Deficient
Vilter, Koch and Spies (6)	Measurement of nicotinamide containing coenzymes I and II by growth of <i>H. influenzae</i> .	0.27-0.87 (43)	0.12-0.54 (45)
Lwoff <i>et al.</i> (7, 8)	Measurement of total nicotinamide as the growth factor for <i>B. proteus</i> .	0.62-0.89 (9)	0.73-1.03 (10)
Kohn, Bernheim and Felsoranyi (9)	Measurement of coenzymes I and II as growth factor for <i>H. parainfluenzae</i> .	0.32-0.8† (53)	0.4 -0.64† (9)
von Euler and Schlenk (10)	Measurement of coenzyme I by yeast fermentation method.	Av. 0.80† (7)	
Axelrod, Gordon and Elvehjem (11, 12)	Same	0.33-0.72† (17)	0.63† (1)
Swaminathan (13)	Chemical determination of total nicotinic acid.	0.33-0.53 (3)	
Ritsert (14)	Same	0.33-0.46 (7)	
Kühnau (15)	Same	0.25-0.45 (7)	0.08-0.18 (4)
Villela (16)	Same	0.25-0.64	

\* The figure in parentheses represents the number of subjects in that study.

† These values were originally given as the coenzyme concentration. The nicotinic acid content of coenzymes I and II is considered to be 17.4 per cent of the molecules (average value) and 18.5 per cent of coenzyme I alone. Whenever originally expressed in terms of corpuscular volume, values were converted to whole blood values by assuming an hematocrit of 43 per cent. All of the coenzymes are in the corpuscles (17) but the hematocrits of patients may have been low.

Most of the workers did not find a significant difference in the nicotinic acid content of the blood of normal and deficient subjects. In the large series of Vilter, Kock and Spies (6), consisting of 43 normal and 45 deficient subjects which showed the most impressive differences, there were 12 in-

<sup>1</sup> The expense of this investigation was defrayed by grants from the Upjohn Company, Kalamazoo, and from the Horace H. Rackham School of Graduate Studies, University of Michigan, Ann Arbor.

<sup>2</sup> Upjohn Fellow in Clinical Research, 1937-1940.

<sup>3</sup> Upjohn Fellow in Clinical Research, 1938-1940.

<sup>4</sup> Upjohn Fellow in Clinical Research, 1940.

stances of overlapping of values for whole blood in the two groups. There were many more instances of overlapping of values between the two groups when they were expressed in terms of the coenzyme content of the blood cells, as was done by Kohn, Bernheim, and Felsoranyi (9), because the coenzymes had been shown to be confined to the corpuscles (17).

Significant decreases in the coenzyme content of the blood of deficient subjects were not found by the other workers who used biological methods. Lwoff and associates (7, 8) measured the growth-promoting effect for *B. proteus*, while Kohn and Bernheim (9) measured that for a strain of *H. parainfluenzae*. When the growth of an organism is used to measure a vitamin, it is necessary to eliminate the effect of all other growth-promoting factors, as was shown by Sinclair (18) in using the growth of *Phycomyces blakesleeanus* as a measure of thiamine. Vilters, Koch and Spies (6) note that parallel studies using both *H. influenzae* and *H. parainfluenzae* have not always given comparable results.

The figures obtained by chemical tests on human blood were lower than those obtained by biological methods and there were few such observations on the blood of deficient subjects. In the chemical methods used, there were possibilities of loss of nicotinic acid by inadequate extraction or by adsorption on charcoal or on precipitated protein. Protein precipitants have been shown to precipitate the nicotinamide-containing coenzymes of the blood (19, 20). Benzene extraction (15, 16) is unreliable because of the very limited solubility of nicotinic acid in this solvent (21).

Pearson (22), using a chemical method, reported the finding of a low blood nicotinic acid in one dog with blacktongue. This was not confirmed by the more extensive studies of blacktongue by Kohn, Klein and Dann (23) or by Axelrod, Madden and Elvehjem (24), using biological methods. It has been a consistent finding (23, 24, 25, 26) that, in blacktongue, there are considerable decreases in the nicotinic acid (or coenzyme) content of some tissues, notably liver and muscle.

In urine, nicotinic acid occurs as its amide, as nicotinuric acid, the glycine conjugate, and as trigonelline, the methylated form. The chemical relationship between these compounds is indicated

by the structural formulae presented in Figure 1. Free nicotinic acid and nicotinuric acid are not present in the urine in significant amounts either normally or after dosage with nicotinamide (5, 27). They both occur in the urine after large doses of nicotinic acid (5, 27). Nicotinamide and nicotinuric acid react directly with the cyanogen bromide and aromatic amine reagents, the intensity of the color produced being dependent upon the choice of the latter. Only after acid or alkaline hydrolyses (preferably acid hydrolysis, to which trigonelline is resistant) of proper normality and duration can theoretical values be obtained for them (28).

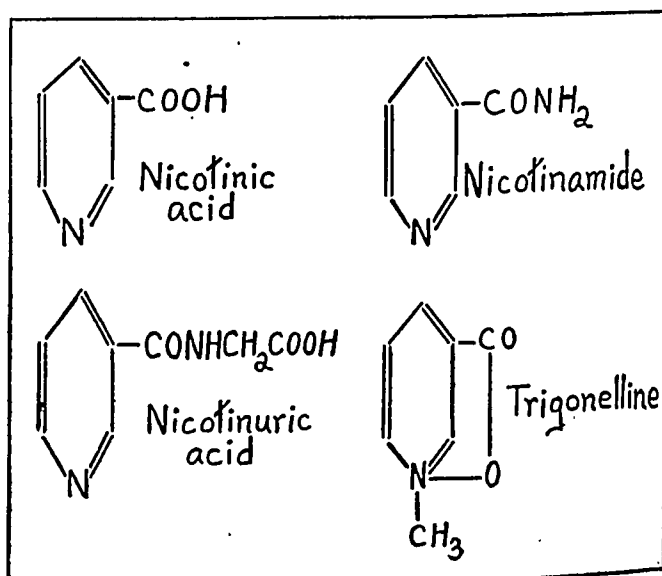


FIG. 1. STRUCTURAL FORMULAE OF NICOTINIC ACID AND ITS DERIVATIVES

The greater portion of nicotinic acid is excreted as trigonelline (27). This does not react with the reagents to produce a color. It is susceptible to alkaline hydrolysis which permits at least partial, semiquantitative recovery (28, 29). There may be, however, large amounts of trigonelline in urine from other sources than methylation of nicotinic acid. It is widely distributed among foods (30, 31), with no quantitative data available except that coffee is a rich source (32). It, or a compound with similar hydrolytic behavior, also occurs from detoxification of nicotine and possibly of other pyridine compounds (28). (A fraction of nicotine, the size of the fraction varying in different individuals, is also excreted in a form which is determined in the "nicotinic acid" fraction of urine.) Since trigonelline possesses no

TABLE II  
*Studies of the excretion of "nicotinic acid" in urine from human subjects*

Author (Reference)	Hydrolytic procedure	Decolorization	Blank test	Reagents for chemical reaction	Urinary "nicotinic acid" values*		Urinary "nicotinic acid" values*		
					Basal		After oral test dose†		
					Normal	Deficient	Dose	Normal	Deficient
					mgm. per 24 hours		mgm.	mgm. per first 24 hours	
Vilter, Spies and Matthews (3)	None	Charcoal	On reagents alone	2,4 dinitrochloro- benzene and sodium hydrosulfide	(7) 20-50	(3) 0			
Bandier (34)	1.2 N NaOH ½ hour	Acetone ex- traction after salting out	Test solution plus cyanogen bromide	Cyanogen bromide and metol	(10) 1.5-5.0		90‡	(1) 12.5	
Swaminathan (35)	2 N NaOH 3 hours	Charcoal	Test solution plus aniline	Cyanogen bromide and aniline	(26) 0.6-8.9	(8) 0.9-2.2	100	(26) 1.1-29.6	(8) 1.0-12.7
Ritsert (14)	2.5 N HCl 2 hours	Benzene ex- traction after dehydration	On reagents alone	Cyanogen bromide and aniline	(8) 0.8-4.5§				
Harris and Raymond (36)	0.8 N NaOH ½ hour	None	Test solution plus p-amino- acetophenone	Cyanogen bromide and p-amino- acetophenone	(6) 3.1-7.3	(2) 1.8-2.9			
Porjé (37)	None	None	On test solu- tion alone	Cyanogen bromide and aniline	(7) 1.2-20.0	(1) 0	50	(7) Approximately 4	(1) 0
Kühnau (15)	2.5 N HCl 2 hours	Benzene ex- traction after dehydration	On reagents alone	Cyanogen bromide and aniline	(7) 0.8-4.5§	(4) 1.0-1.6§	100-500	(7) 0-4	(2) 0-4
Rosenblum and Jolliffe (38)	0.08 M KH <sub>2</sub> PO <sub>4</sub> 5 minutes	None	Test solution plus cyanogen bromide	Cyanogen bromide and metol	(13) 3.4-10.2	(1) 0-2.8	100		(1) 1

\* The figure in parentheses represents the number of subjects used in each of the studies.

† These values have been corrected for the basal excretions prior to the administration of the test dose.

‡ Test dose of nicotinic acid taken while fasting.

§ These values, given originally by the authors as *micrograms per cent*, were obtained by assuming an average 24-hour urine volume of 1500 cc.

|| In these tests nicotinamide constituted the test dose.

anti-blacktongue activity (33), provision must be made to avoid the obscuration of results by the excretion of trigonelline arising from non-vitamin sources.

In Table II are summarized the analyses of urines from both normal and pellagrous subjects reported from other laboratories. In order to evaluate the data, it is necessary to consider the procedure used, as will be noted below. Those using the more sensitive cyanogen bromide reaction rarely found an absence of nicotinic acid in the urine. Harris and Raymond (36) Kühnau (15) and Rosenblum and Jolliffe (38), on the basis of a small number of observations, considered that the difference in the urinary nicotinic acid in normal and deficient subjects was significant. Swaminathan (35) reported a marked overlapping of the values for the two groups, although he did find a decreased average secretion in the deficient group. As has been noted, the chemical methods are not specific for nicotinic

acid and its derivatives. Much of the difference in the urinary "nicotinic acid" of the normal and deficient groups may have been due to smoking by the former group.

Attention to the different procedures used by those employing the cyanogen bromide and aromatic amine reagents indicates that the nicotinic acid compounds included in the reported "nicotinic acid" values differ in the various studies. This will depend upon the type of hydrolysis used, the method for decolorization and evaluation of residual color, and the aromatic amine employed. Bandier (34) and Harris and Raymond (36) must have measured all of the nicotinic acid, nicotinamide and nicotinuric acid, and possibly a slight amount of trigonelline. Swaminathan's (35) values included, in addition, an appreciable fraction of the trigonelline. Ritsert's procedure (14) should measure the nicotinic acid, its amide, about two-thirds of the nicotinuric acid (because of the color which it, unhydrolyzed, produces with the

reagents used (28)) and no trigonelline. The procedure used by Porjé (37), involving no preliminary hydrolysis, should measure any nicotinic acid present, about one-half of the nicotinamide, two-thirds of the nicotinuric acid (by direct reaction with the reagents) and no trigonelline. Rosenblum and Jolliffe's values (38), because of the slight preliminary hydrolysis and the aromatic amide used, presumably include 142 per cent of the nicotinamide and 42 per cent of the nicotinuric acid (values representing the intensities of color produced by these unhydrolyzed molecules with the aromatic amine used relative to those produced by nicotinic acid).

In addition to the differences in what constituted the urinary "nicotinic acid" values, as indicated above, there are other possible discrepancies inherent in the methods listed in Table II. When the test solution is analyzed without preliminary decolorization (36, 37, 38), the increment of color produced constitutes a small fraction of the total color measured. The use of *direct* charcoal decolorization (5, 35) permits loss of nicotinic acid by adsorption (39). As noted above, benzene extraction (14, 15) is unreliable because of the limited solubility of nicotinic acid in benzene (21). In the blank test for evaluating residual color, as used by Bandier (34) and by Rosenblum and Jolliffe (38), nicotinic acid reacts directly with cyanogen bromide to give some of the test color even in the absence of the aromatic amine (39). To include in the blank the color developed by the addition of aniline to the hydrolysate has also been shown to be erroneous, since it is due to a reaction of aniline with other substances which does not occur in the presence of cyanogen bromide (40).

Fraser, Topping and Sebrell (41), using a bacterial growth method with *Shigella paradysenteriae*, reported a decrease in the urinary excretion of nicotinic acid (or its related compounds) in dogs with blacktongue. In a subsequent paper (25) from the same laboratory, using the *Haemophilus parainfluenzae*, no such difference in the urinary excretion values was reported. Harris and Raymond (36) observed a decrease in the urinary excretion of nicotinic acid (chemically determined) in guinea pigs on an inadequate diet only after marked weight losses and within a week

before death. In the case of the sheep, Winegar, Pearson and Schmidt (42) were unable to find any decreases in nicotinic acid excretion.

#### EXPERIMENTAL

We have studied the possibility of the chemical diagnosis of nicotinic acid deficiency by simple analyses of urine and blood and by a variety of "saturation tests."

An application of the cyanogen bromide and aniline reagents, which we have described (39, 40, 28) was used for the determination of nicotinic acid and its compounds. It is designed to avoid errors, which are due to inadequate extraction or to adsorption, by direct hydrolysis of the test substance followed by *preferential* charcoal adsorption for the decolorization of the hydrolysate. By this method the blood nicotinic acid values are higher than those reported by other chemical methods and fall within the range of the values obtained by bacterial growth or enzyme fermentation methods (Table I). Nicotinamide and coenzyme I added to blood have been found to be completely hydrolyzed and recovered as free nicotinic acid (4).

*Studies of the blood and plasma nicotinic acid content.* The whole blood nicotinic acid values of 17 patients before treatment are presented in Table III. In some of them, the nicotinic acid content of the plasma was also determined. The figures obtained fall well within the range of those found in 40 normal subjects.

TABLE III  
*Comparison of the blood nicotinic acid values of normal subjects and of nicotinic-acid-deficient patients before treatment*

Case number	Sex	Degree of deficiency	Whole blood values	Plasma value
			mgm. per cent	mgm. per cent
1	F	Marked	0.66	0.15
2	F	Marked	0.49	
4	F	Marked	0.94*	
5	F	Marked	0.77	
8	F	Marked	0.81	
9	F	Marked	0.61	0.14
10	M	Marked	0.69	0.26
11	F	Moderate	0.55	0.00
12	F	Moderate	0.64	
13	M	Moderate	0.75	
14	F	Moderate	0.67	
15	F	Moderate	1.00	
17	F	Suboptimal	0.87	0.13
18	F	Suboptimal	0.64	
19	F	Suboptimal	0.57	
21	M	Suboptimal	0.71	
22	M	Suboptimal	0.56	
Normal controls (40 subjects)†				(10 subjects)
Average			0.67	0.15
Range			0.52-0.83	0.05-0.24

\* This patient had an hematocrit of 52 per cent.

† The data on the normal blood and plasma nicotinic acid values are presented elsewhere (4).

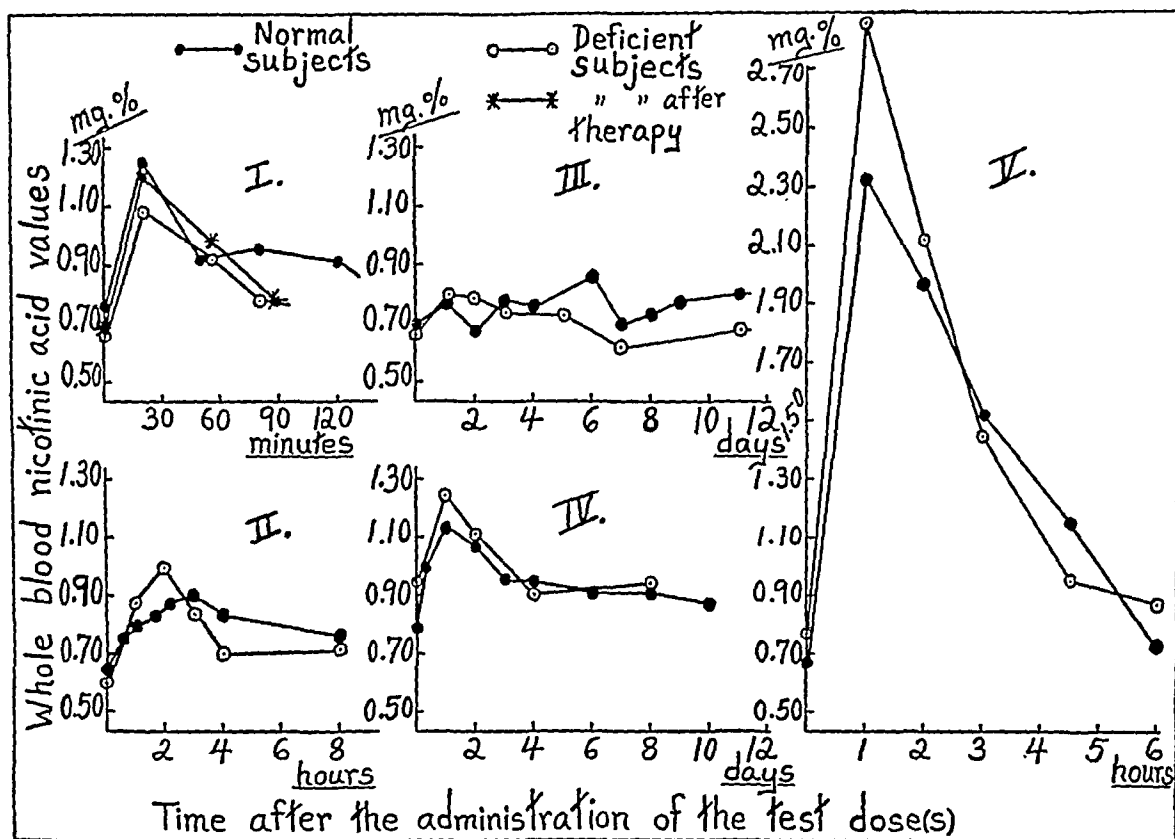


FIG. 2. TYPICAL BLOOD NICOTINIC ACID AND NICOTINAMIDE TOLERANCE CURVES OBTAINED WITH NORMAL AND NICOTINIC-ACID-DEFICIENT SUBJECTS

Graph I. Following the intravenous administration of nicotinamide, 5 mgm. per kilogram of body weight.

Graph II. Following the oral postprandial administration of 500 mgm. of nicotinic acid.

Graph III. During the postprandial ingestion of 200 mgm. of nicotinamide, three times daily. Each blood sample was taken at least 12 hours after the last dose.

Graph IV. Following the oral administration of nine 200 mgm. doses of nicotinic acid, one every 2 hours. (The values are plotted in days after the ingestion of the first of the test doses.)

Graph V. Following the intramuscular administration of nicotinamide, 500 mgm. per square meter of surface area.

A large number of "tolerance curves" of blood nicotinic acid values were obtained following the administration of nicotinic acid or nicotinamide to normal and deficient subjects. Since the data are voluminous and the results are negative, only the curves obtained with one normal subject and with one definitely deficient patient are presented for each of five different procedures (Figure 2). All the tests have been confirmed by similar studies with other normal and deficient subjects.

In Graph I of the figure are plotted the serial blood values obtained following the intravenous administration of nicotinamide, 5 mgm. per kilogram of body weight (equivalent to about nine times the total quantity, free and combined, of nicotinic acid in the blood). In no case of nicotinic acid deficiency was there any significant difference from the normal in the rate of removal of the amide from the blood stream. Even when the test was

repeated with the same patient, shortly after 2 weeks of intensive nicotinamide therapy, no appreciable change in the tolerance curve was noted. The relatively small increase in the blood nicotinic acid and the prompt return to the basal level, which was noted in every case, could not be explained by rapid excretion of the compound. Only 10 per cent of the test dose was found in the urine.

The serial blood values obtained following the oral postprandial administration of 500 mgm. of nicotinic acid (Graph II) likewise showed no significant difference between the normal and deficient subjects. In this type of test there is a slower increase in the blood nicotinic acid concentration, the maximum being attained from 2 to 3 hours after the test dose is taken. The maximal values, however, are much smaller than those recorded in Graph I following intravenous administration.

In Graph III are presented typical blood values obtained during the postprandial administration of 200 mgm. of nicotinamide three times daily over a period of 11 days. Each blood sample was taken at least 12 hours after the last dose. No significant differences were observed between the blood values obtained with the normal and deficient subjects. In no case were there significant variations from the initial basal blood values.

It was thought that possibly in the above tests the increases in the blood values were not sufficiently large or prolonged to allow differences in the rate of removal of nicotinic acid (or amide) from the blood to be noted. In Graph IV the serial blood values following the oral administration of nine 200 mgm. doses of nicotinic acid, one every 2 hours, are plotted. In these tests the elevated blood values persisted over an appreciable period of time (more than 7 days) but, again, differences between the responses of the normal and deficient subjects were not apparent.

In Graph V are presented representative data following the intramuscular administration of nicotinamide, 500 mgm. per square meter of surface area. In these tests, markedly elevated blood values were obtained and these persisted for a considerable period of time (more than 3 hours). However, no differences were observed between the blood values obtained with normal and deficient subjects.

Extensive studies involving serial plasma nicotinic acid determinations were not carried out. Preliminary investigations (4) had shown that the plasma values return to the basal level even more promptly than those for whole blood.

*Studies of the urinary excretion of nicotinic acid and its derivatives.* In Table IV are presented the results of analyses of 24-hour urine specimens from normal subjects and from patients with severe dietary deficiency. Four of the patients had fully developed "classic" pellagra. Case 3 had alcoholic neuritis with a mildly red-tened tongue and no significant skin changes. The other

2 patients had pyloric or intestinal obstruction and had eaten very little for a considerable time but had only minor skin changes.

During the time of collection of the specimens, and usually for one or more days before, the subjects abstained from the use of tobacco, coffee, tea and chocolate. Preliminary observations have indicated that the latter two substances affect the results very little. The use of coffee and tobacco, as noted above, causes large increases in the trigonelline and, in some individuals, in the apparent nicotinic acid excretion; these increases are unrelated to the vitamin metabolism. The avoidance of them is a minimal requirement for a basal regime which will permit chemical analyses of urine by present methods to have any significance in relation to nicotinic acid nutrition.

It will be seen that the apparent nicotinic acid excretion of the deficient subjects tended to be less than that of the normal subjects, averaging 2.7 mgm., against 3.8 mgm. per 24 hours. There were, however, several instances of overlapping of values between the two groups. It is quite possible that this overlapping was due to the excretion of other ingested pyridine compounds and might not occur with a more specific method of analysis.

The differences between the trigonelline excretions of the deficient and the normal subjects were must greater; an average value of 3.1 mgm. per 24 hours for the deficient group compared with 19.5 mgm. per 24 hours for the normal group. They were also much more consistent. The one patient whose trigonelline excretion approximated the lowest value obtained in any normal subject, and who had alcoholic neuritis, was a non-smoker. The analysis was done so long after the collection of the specimen that it was impossible to determine if the coffee had been omitted.

## DISCUSSION

We had found that, in a dog, the blood nicotinic acid remained elevated for many hours after the intravenous injection of a sufficiently large dose of nicotinic acid. The doses required were large—10 mgm. per kilo. Giving comparable parenteral doses of nicotinic acid to humans was not feasible but we had found that such parenteral doses of nicotinamide produced no unpleasant symptoms in ourselves other than some local pain, when given intramuscularly. Because it had been demonstrated that the tissues of experimentally deficient animals had a decreased content of nicotinic acid-containing enzymes, it was thought possible that nicotinic acid and nicotinamide would be removed from the blood of deficient subjects faster than from that of normal subjects. Such does not appear to happen. We have found no indication that the blood nicotinic acid content,

TABLE IV

*Urinary excretion during abstinence from coffee, tobacco, tea and chocolate*

Normal subjects			Deficient subjects		
Case	Nicotinamide	Trigonelline	Case	Nicotinamide	Trigonelline
	mgm. per 24 hours	mgm. per 24 hours		mgm. per 24 hours	mgm. per 24 hours
M. E. B.	3.4	12.0	1	1.3	3.9
M. E. B.	4.1	13.8	2	3.6	1.5
M. E. B.	5.5	12.3	2	3.3	3.3
M. E. B.	4.1	9.0	3	4.2	7.5
H. G. W.	3.1	10.2	4	3.6	1.2
	3.5	16.8	5	1.7	3.0
	3.6	15.8	6	2.8	3.3
			7	1.2	0.9
15 other specimens from 8 subjects	2.8-6.2	6.9-42.0	7	2.6	3.3
Average	3.8	19.5	Average	2.7	3.1

either under basal conditions or after any type of dosage, is a measure of nicotinic acid nutrition.

This is unfortunate because the present chemical methods for determination of nicotinic acid are not specific. Other pyridine compounds present in vegetable materials give reactions similar to those of nicotinic acid and the methylated form, trigonelline, in which the greater part of nicotinic acid is excreted in the urine. It is possible that a more specific method would show more distinctive differences in the urinary excretion of nicotinamide.

In order to make determinations of trigonelline excretion significant as a measure of nutrition, it is necessary to have subjects on a basal regime eliminating at least the major sources of trigonelline of non-vitamin origin—coffee and tobacco. What further standardization of the basal regime is desirable remains to be determined. It may be that part of the decrease in the trigonelline excretion of the deficient subjects was due to a lesser intake of preformed trigonelline in a smaller intake of all foods. It appears that, with a sufficiently standardized basal regime and with a satisfactorily quantitative method of recovery of trigonelline, its excretion in the urine may be a measure of nicotinic acid nutrition.

#### SUMMARY

1. A review of the literature has shown no agreement as to the value of determination of the nicotinic acid compounds in urine or blood as a measure of nicotinic acid nutrition.
2. The data of the earlier reports have been re-assessed, in the light of more recent knowledge, to indicate what compounds were included in the reported "nicotinic acid" values, and the validity of methods has been discussed.
3. In the present study, no differences were observed in the basal blood or plasma nicotinic acid content of normal and deficient subjects.
4. Five different types of blood nicotinic acid or nicotinamide tolerance curves showed no difference between normal and deficient subjects.
5. With the procedures used in this study there was an average, but not a constant, decrease in the urinary excretion of substances reacting like nicotinamide by deficient subjects.
6. There was a more marked and a more consistent decrease in the urinary excretion of tri-

gonelline in the deficient subjects. With a sufficiently standardized basal regime and with a satisfactorily quantitative method of recovery of trigonelline, the urinary excretion of trigonelline may be a measure of nicotinic acid nutrition.

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# VITAMIN A AND CAROTENE. I. THE DETERMINATION OF VITAMIN A IN THE BLOOD AND LIVER AS AN INDEX OF VITAMIN A NUTRITION OF THE RAT

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Considerable interest has been evidenced during the past few years in the attempt to correlate the blood vitamin A content of the human under various conditions with the nutritional status of the body (1 to 6). In these studies, normal ranges were determined by estimation of the blood vitamin A for a large number of healthy subjects. In some instances (1, 4), human subjects were placed on vitamin A-deficient diets and the blood levels were followed over periods of two to six months. It was not possible to give diets entirely deficient in vitamin A, or to continue the diets for sufficient lengths of time to reach critically low vitamin A levels. Because human subjects were used, it was also impossible to correlate the blood values with the liver stores. The present study, using the rat as the experimental animal, was designed to aid in the interpretation of data obtained on human subjects. Blood and liver vitamin A concentrations were determined on rats during the course of depletion and while they were fed definite quantities of vitamin A.

## EXPERIMENTAL

Eighty-six rats from a Wistar strain, twenty-eight days old, were placed on the basal diet given in Table I. Group I remained on the basal diet throughout the experiment. All animals in group II received the basal diet plus four units of vitamin A per day until the thirty-fifth day. At this point it was found that the body stores had been depleted. A part of this group was then reduced to zero units per day, a part remained on four units per day, and a part was raised to twenty units per day for the remainder of the experiment. Group III was depleted until loss of weight and incipient xerophthalmia indicated avitaminosis A. At this point each animal was given twenty units per day throughout the remainder of the experiment. Group IV was depleted in the same manner as group III. At the end of depletion each animal was given 1200 units of vitamin A as a single dose, representing twenty units for each day on the depletion

<sup>1</sup> The expense of this study was defrayed in part by a grant from the Horace H. Rackham and Mary A. Rackham Foundation.

diet, and twenty units per day each day thereafter until the end of the experiment.

Throughout the experiment, groups of three, four, or five rats, depending on their size, were killed for analysis of the blood and liver at appropriate intervals. The pooled blood from each group was allowed to clot and the serum removed. Vitamin A determinations were run according to Kimbles' (5) modification of the method of Dann and Evelyn (7), using the macro unit of the Evelyn photoelectric colorimeter. The livers of each group of

TABLE I  
Basal diet

	Total weight	Total calories
	per cent	per cent
Casein, vitamin free.....	18	16
Corn starch.....	63	55
Salts (Osborne and Mendel (1919))..	4	
Hydrogenated vegetable oil (Crisco)	15	29
	100	100

Vitamins B, G, and D were supplied daily in the form of pellets containing 400 mgm. of dried brewer's yeast and 10 I.U. of vitamin D as irradiated Ergosterol. When a supplement of vitamin A was given, biologically standardized cod liver oil, diluted to furnish the desired number of International Units of vitamin A, was added to the pellets.

rats were similarly combined and weighed. They were then ground with anhydrous sodium sulfate until a fine powder was obtained. After making up to 100 cc. with water, and shaking well, 5 cc. aliquots of this viscous suspension were taken for analysis. Two cc. of 30 per cent NaOH were added to these samples, after which they were heated in a water bath under nitrogen for an hour, this being considered sufficient time to hydrolyze the esters of vitamin A in the liver. The vitamin A in the hydrolysate was then determined in a manner analogous to that applied to the blood samples.

The concentrations of vitamin A in blood and liver are expressed as micrograms per 100 cc. of serum or 100 grams of liver tissue. To do this, the *L* (1 cm., 1 per cent, 620  $m\mu$ ) values obtained on the Evelyn colorimeter and calculated according to Dann and Evelyn (7) are converted into the spectrophotometric extinction coefficient *E* (1 cm., 1 per cent, 328  $m\mu$ ). The conversion factor, as determined by Dann and Evelyn, is *E* (1 cm., 1 per cent, 328  $m\mu$ ) = *L*  $\times$  (0.41  $\pm$  0.05). Holmes and Corbet (8) have succeeded in preparing crystalline vitamin A

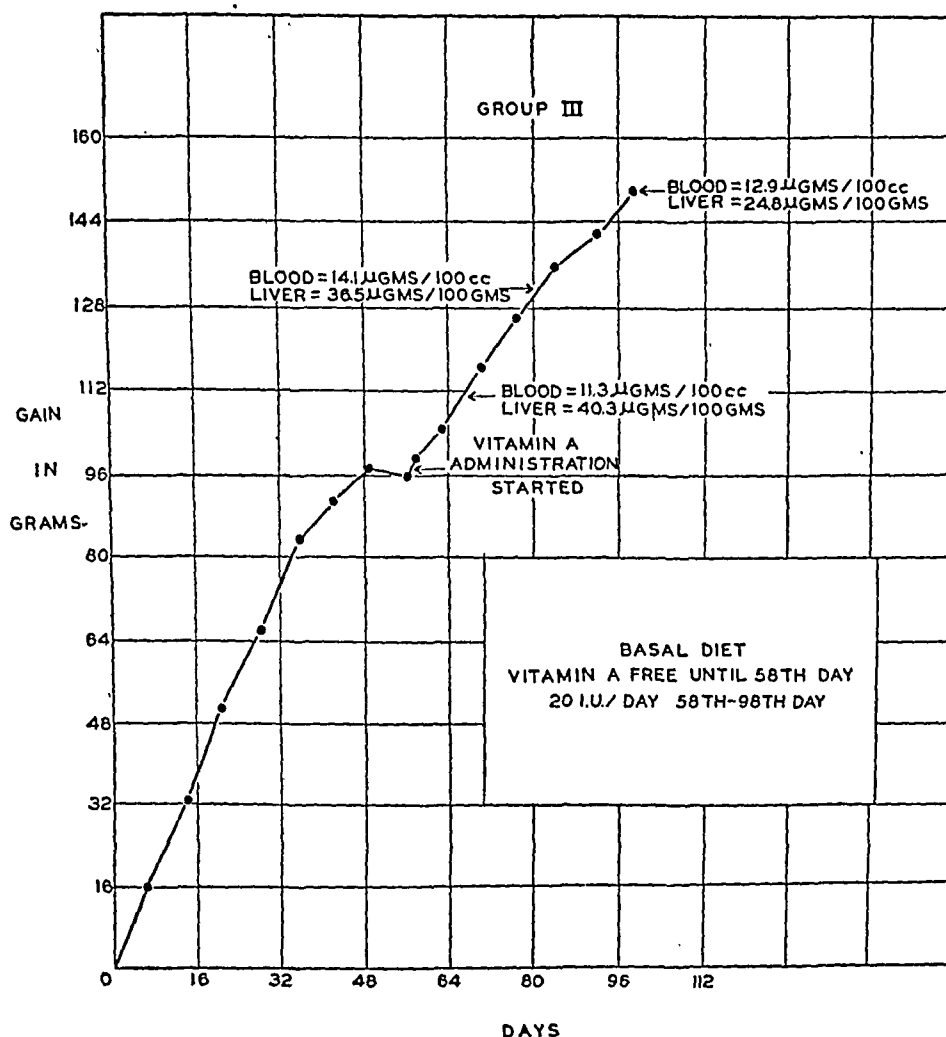


FIG. 3. GROWTH CURVE AND CONCENTRATIONS OF VITAMIN A IN THE BLOOD AND LIVER OF RATS RECEIVING NO VITAMIN A UNTIL THE FIFTY-EIGHTH DAY, THEN TWENTY I.U. PER DAY THEREAFTER UNTIL THE END OF THE EXPERIMENT

min A until growth ceased. At this point it was assumed that their blood and livers were depleted of vitamin A, as were those of the animals receiving an initial supplement of four I.U. per day, mentioned above. A daily supplement of twenty I.U. of vitamin A was begun and immediately normal growth was resumed. Here again the response is shown by a rise in the blood vitamin A, but there is little change in the liver. Apparently, twenty I.U. of vitamin A per day are sufficient to keep the blood concentrations high enough to maintain body functions but do not furnish enough to allow storage in the liver.

Of the rats receiving four I.U. of vitamin A per day until the thirty-fifth day (Figure 2), part were continued with the same dosage until the end of the experiment. The growth of these animals began to fall off by about the forty-ninth day. Though they did not actually lose weight, they did

little more than maintain themselves. When they were killed on the ninety-first day, the livers were completely empty, as would be expected from the analysis of the livers on the thirty-fifth day.

The third curve in Figure 2 represents animals from whom vitamin A was taken away altogether on the thirty-fifth day, after they had received four I.U. per day until this time. These rats were killed on the eighty-fourth day after loss of much weight and, as was expected, there was no vitamin A in the blood or liver. These animals had lost so much weight and were in such poor condition that they would certainly have died within a week if they had not been killed. It is also unlikely that the controls could have survived long beyond the seventy-seventh day when they were killed for analysis, since members of the group had already died, probably due to infection. Thus, the daily supplement of four I.U. of vitamin A received by

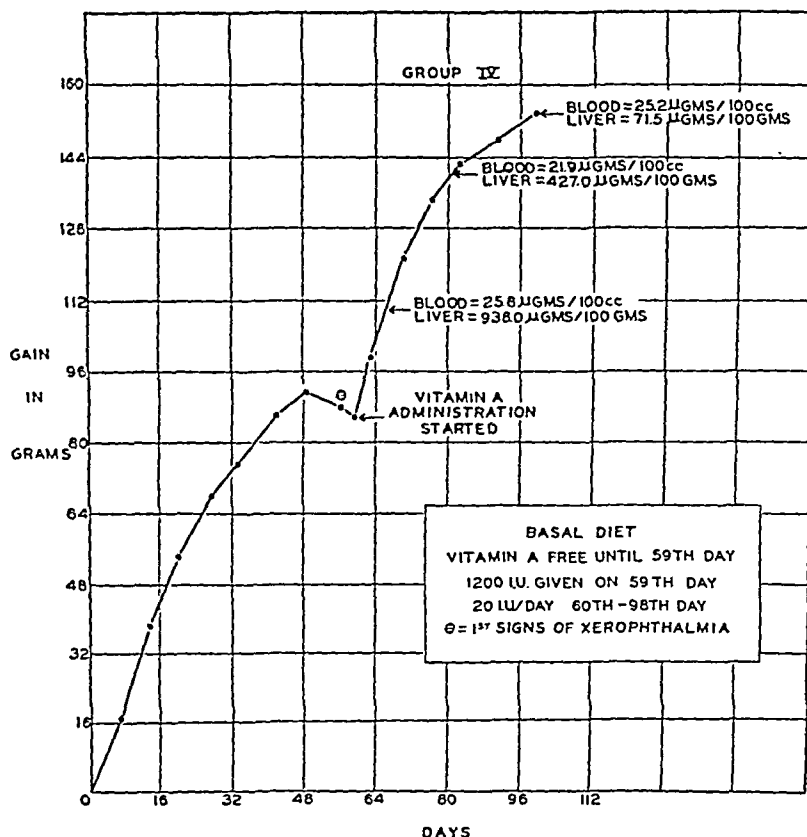


FIG. 4. GROWTH CURVE AND CONCENTRATIONS OF VITAMIN A IN THE BLOOD AND LIVER OF RATS RECEIVING NO VITAMIN A UNTIL THE FIFTY-NINTH DAY, THEN 1200 I.U. AS A SINGLE DOSE AND 20 I.U. PER DAY THEREAFTER UNTIL THE END OF THE EXPERIMENT

these rats for thirty-five days did not appear to prolong their lives beyond those of the controls who had received no supplement at all. Apparently, four I.U. of vitamin A daily had not slowed down depletion of the body stores appreciably. This is surprising since, by definition, one Sherman unit (approximately 1.4 I.U.) is sufficient to cure xerophthalmia and to produce slight growth. Anything above two I.U. has been generally accepted as enough to produce normal growth. Baumann, Riising, and Steenbock (10) found that as little as one I.U. was sufficient to cure xerophthalmia and restore growth but that seven to seventeen I.U. were required to produce storage in the liver. Goss and Guilbert (11) state that eighteen to twenty-two I.U. per day per kgm. (about 1.8 to 2.2 I.U. for young rats) are necessary to prevent vaginal cornification, but they found no storage of the vitamin in the liver if less than 382 I.U. per day per kgm. were given. We have shown in

other experiments (12) that four I.U. daily are sufficient to cure xerophthalmia and cause the rat to resume normal growth at least for a short period, but unreported determinations on these rats show that this amount does not raise the concentration in the blood above the depletion level. The present experiment shows that four I.U. are enough only to maintain body weight and protect against xerophthalmia, but furnish no vitamin A to the blood or liver as a margin of safety for the animal.

Figure 4 presents the growth curve for twelve rats which, at the end of the depletion period, received 1200 I.U. of vitamin A as a single dose, representing twenty I.U. per day for each day on the depletion diet, and thereafter received twenty I.U. per day. Here again normal growth was resumed. Rats killed seven days after the initial administration showed a large liver reserve and a normal blood value. Sixteen days later, however,

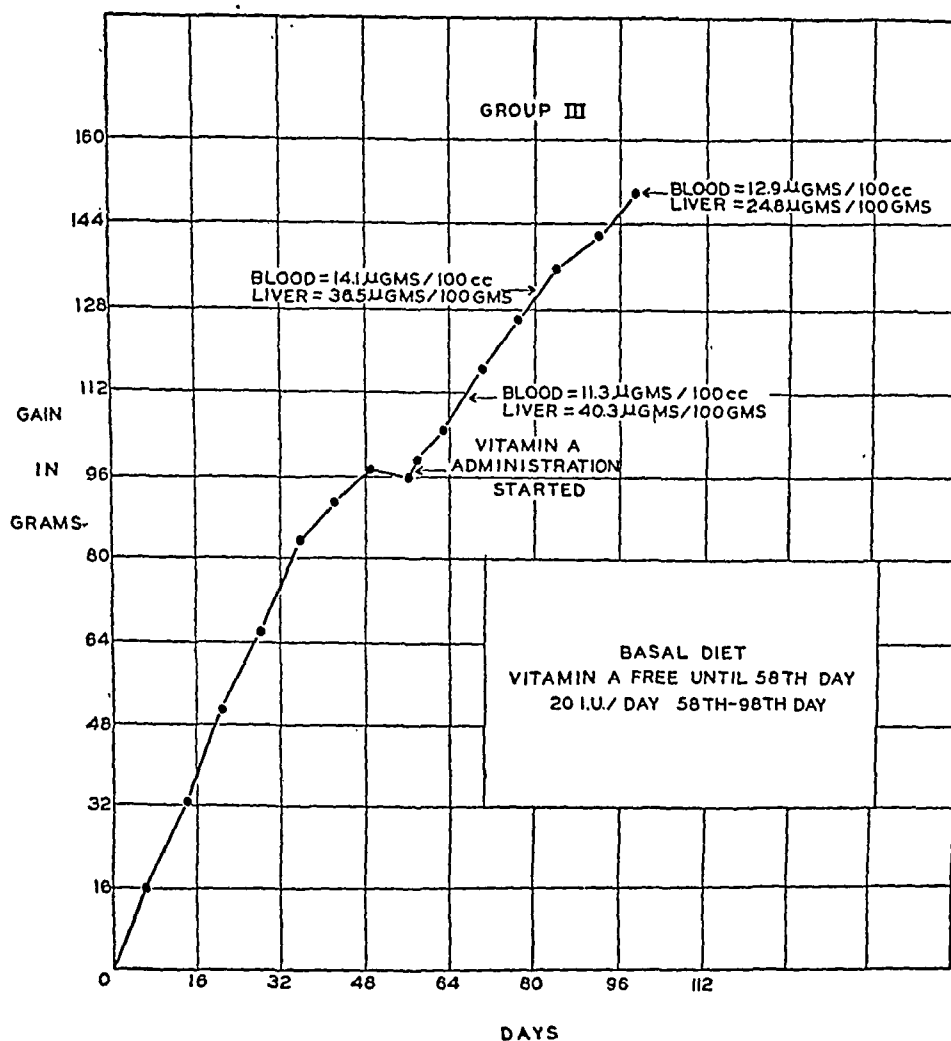


FIG. 3. GROWTH CURVE AND CONCENTRATIONS OF VITAMIN A IN THE BLOOD AND LIVER OF RATS RECEIVING NO VITAMIN A UNTIL THE FIFTY-EIGHTH DAY, THEN TWENTY I.U. PER DAY THEREAFTER UNTIL THE END OF THE EXPERIMENT

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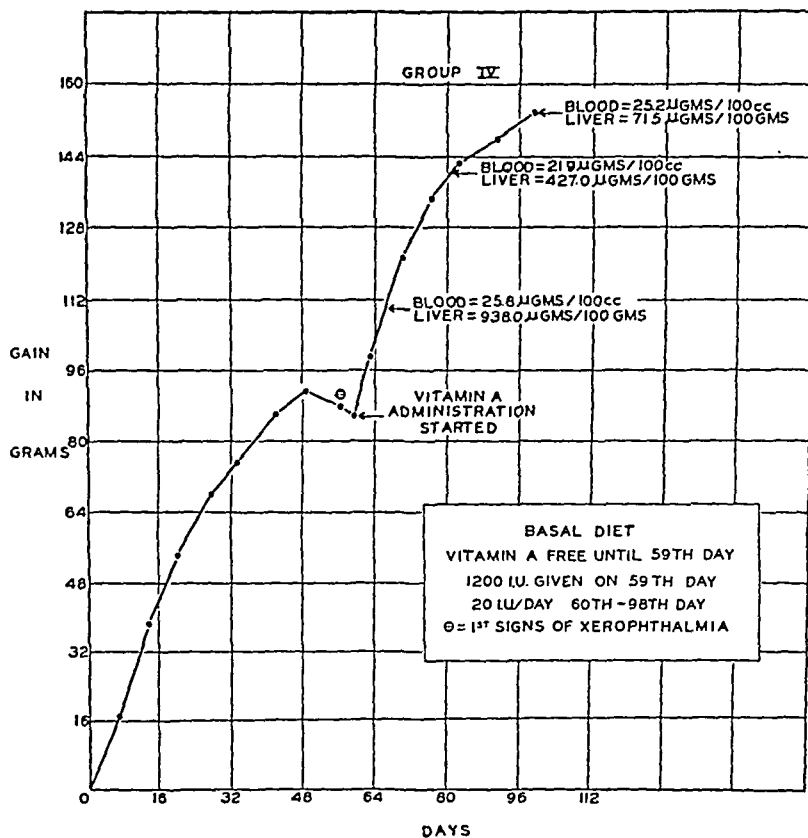


FIG. 4. GROWTH CURVE AND CONCENTRATIONS OF VITAMIN A IN THE BLOOD AND LIVER OF RATS RECEIVING NO VITAMIN A UNTIL THE FIFTY-NINTH DAY, THEN 1200 I.U. AS A SINGLE DOSE AND 20 I.U. PER DAY THEREAFTER UNTIL THE END OF THE EXPERIMENT

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Figure 4 presents the growth curve for twelve rats which, at the end of the depletion period, received 1200 I.U. of vitamin A as a single dose, representing twenty I.U. per day for each day on the depletion diet, and thereafter received twenty I.U. per day. Here again normal growth was resumed. Rats killed seven days after the initial administration showed a large liver reserve and a normal blood value. Sixteen days later, however,

TABLE II  
Summary of blood and liver determinations

	Vitamin A				
	Blood		Liver		Liver fat
	<i>L</i> (1 per cent 1 cm.) × 10 <sup>3</sup>	micro-grams per cent	<i>L</i> (1 per cent 1 cm.) × 10 <sup>3</sup>	micro-grams per cent	grams per cent
Group I—depleted					
0 days.....	1.44	28.1	10.40	203.0	
7 days.....	1.59	31.0	14.35	280.0	6.25
11 days.....	0.99	19.3	15.06	294.0	8.02
14 days.....	0.59	11.5	3.15	61.5	5.84
18 days.....	0.56	10.9	2.89	56.5	10.05
21 days.....	0.14	2.7	1.12	21.8	13.80
38 days.....	0.09	1.7	1.22	23.8	
77 days.....	0.0	0.0	1.21	23.5	8.30
Group II—Four I.U. vitamin A per day to thirty-fifth day					
Thirty-fifth day.....	0.14	2.7	0.90	17.6	
20 units per day thirty-fifth to sixty-sixth day.....	0.67	13.1	2.01	39.2	
20 units per day thirty-fifth to ninety-eighth day.....			1.20	23.5	
4 units per day thirty-fifth to eighty-fourth day.....			0.77	15.1	11.25
0 units per day thirty-fifth to eighty-fourth day.....	0.16	3.1	1.22	23.8	14.55
Group III—depleted to fifty-eighth day					
20 units per day to sixty-seventh day.....	0.58	11.3	2.06	40.3	
20 units per day to eighty-first day.....	0.72	14.1	1.85	36.1	19.35
20 units per day to ninety-eighth day.....	0.66	12.9	1.27	24.8	17.40
Group IV—depleted to fifty-ninth day—1200 I.U. vitamin A given as one dose					
20 units per day to sixty-sixth day.....	1.32	25.8	48.00	938.0	
20 units per day to eighty-second day.....	1.12	21.9	21.80	427.0	
20 units per day to ninety-eighth day.....	1.29	25.2	3.66	71.5	14.85

the concentration in the liver had gone down by more than one-half, and on the thirty-ninth day after the administration the liver was nearly depleted of vitamin A. The concentration in the blood in each instance remained normal. Throughout this time the liver had been furnishing a decreasing vitamin A supply, but this plus the dietary supplement was sufficient to maintain a normal concentration in the blood. These values indicate that even twenty units per day, though they support normal growth, are not sufficient to maintain the liver stores. At the end of the experiment these animals were little better off than those shown in Figure 3 who had not received the massive dose at the end of depletion. In view of the findings of Baumann *et al* (10), twenty I.U. of vitamin A per day seemed to us a liberal amount. The fact that they were not enough to maintain liver storage was surprising. Work is now in progress on the vitamin A requirement for normal liver storage.

Table II summarizes the material in the charts, giving the vitamin A concentration both in *L* units

and in micrograms per 100 cc. of material used. Determinations on the fat content of the livers are also included. We wished to find out whether there was any change in fat content, paralleling the changes in vitamin A. However, we could find no such parallelism. This confirmed the observations of Thorbjarnarson and Drummond (13).

From the above data, it seems fair to conclude that determinations of the concentration of vitamin A in blood are an index to the vitamin A nutrition of the body in the rat. There is definitely a relationship between the concentration in the blood and the liver stores. A normal blood concentration cannot be maintained unless there is adequate liver storage. As the liver stores are depleted, the concentration in the blood gradually decreases, since intermediate concentrations are found in the blood when the liver stores are low. Complete absence of vitamin A in the blood and in the liver occurs at about the same time. In cases of suboptimal intake, the concentration in the blood is especially significant. Apparently, if it is maintained above a certain minimum, the

tissues are furnished with sufficient vitamin A for continued growth and development. When this concentration cannot be maintained from the diet or liver stores, the animal starts to lose weight and to develop characteristic avitaminosis A symptoms. These, however, occur as very late manifestations of low blood and liver concentrations. Even when the animal has been shown by chemical analysis to be depleted of vitamin A, there is a long and unexplained lag in the physiological response.

#### SUMMARY

1. A total of eighty-six rats, twenty-eight days old, were placed on basal diets containing no vitamin A. Part were given supplements of a definite amount of the vitamin at the beginning of the experiment, and part were given none throughout. The rest were depleted of vitamin A, then divided into groups, each group receiving different amounts of vitamin A. Animals were killed at intervals during the experiment and the vitamin A content of the livers and blood was determined.

2. When no vitamin A was included in the diet, its concentration in the blood and liver declined in a parallel manner. Animals receiving four I.U. per day showed depletion levels in the blood and liver at approximately the same time as those receiving no supplement. Twenty I.U. per day were not sufficient to maintain storage in the liver, but did maintain an elevated (though subnormal) concentration in the blood. These animals grew normally.

3. It is concluded that determinations of the concentration of vitamin A in the blood are an index of the vitamin A nutrition of the body.

Since this paper was accepted for publication, J. M. Lewis and others have published data which for the most part confirm the findings presented here (14).

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# VITAMIN A AND CAROTENE. II. VITAMIN A AND CAROTENE METABOLISM IN DIABETICS AND NORMALS

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Investigation of vitamin A and carotene metabolism in diabetes mellitus has led to several interesting observations by different workers. Ralli, *et al* (1) have shown that the blood level of carotene is higher in the diabetic than in the normal. She also found that the administration of carotene in oil to diabetic patients caused the blood carotene to rise to a higher level and to remain there longer than in the normal controls. From this Ralli postulated that in diabetes there is a diminished ability of the liver to convert carotene to vitamin A. Brazer and Curtis (2) found supporting evidence for this theory in work based on the Jeans biophotometer test. They reported that dark adaptation was abnormally slow in patients with diabetes mellitus, indicating vitamin A deficiency. The administration of carotene to these patients caused no change in the biophotometer reading, whereas vitamin A, in equivalent amounts, quickly returned their dark adaptation time to normal.

Recent work in our laboratory (3), using rats, has shown that the concentration of vitamin A in the blood is a reliable index to the vitamin A status of the body. In view of this, we felt that it was of interest to investigate further the vitamin A of the diabetic individual and to examine the theory postulated by Ralli. This was done by following the blood vitamin A and carotene levels of normals and of patients with diabetes mellitus.

## EXPERIMENTAL AND DISCUSSION

Vitamin A and carotene were determined by Kimble's (4) modification of the method of Dann and Evelyn (5), using the macro unit of the Evelyn photoelectric colorimeter. Vitamin A is expressed as micrograms per 100 cc. of plasma. This is derived from the fundamental *L* (1 per cent, 1 cm., 620  $m\mu$ ) values, calculated according to Kimble (4). By converting *L* (1 per cent, 1 cm.,

620  $m\mu$ ) to the spectroscopic unit *E* (1 per cent, 1 cm., 328  $m\mu$ ) (5), and applying the value for crystalline vitamin A determined by Holmes and Corbet (6), it is possible to express our results as micrograms per 100 cc. Carotene, which is also recorded in micrograms per 100 cc., was calculated from a standard reference curve, using two sources of crystalline carotene (Eastman Kodak Company 3702 and Nutritional Research Associates, Inc.) with color filter 440. By this method, plasma vitamin A and carotene concentrations were determined on a number of healthy controls (physicians, dietitians, nurses, and laboratory workers, all eating in the same dining room). The blood on which the

TABLE I  
Carotene and vitamin A blood values of normal subjects

Males				Females					
Number	Carotene	Vitamin A		Number	Carotene	Vitamin A			
	<i>micrograms per cent</i>	<i>L units</i> $\times 10^3$	<i>micrograms per cent</i>		<i>micrograms per cent</i>	<i>L units</i> $\times 10^3$	<i>micrograms per cent</i>		
1	250	1.90	37.1	1	250	1.70	33.2		
2	310	2.10	41.0	2	230	1.40	27.3		
3	160	1.70	33.2	3	210	1.30	25.4		
4	210	2.10	41.0	4	280	1.50	29.2		
5	110	1.30	25.4	5	170	1.30	25.4		
6	130	1.40	27.3	6	220	1.40	27.3		
7	140	2.00	39.0	*7	420	1.90	37.1		
8	110	1.40	27.3	8	250	1.40	27.3		
9	230	1.40	27.3	9	240	1.60	31.2		
10	200	1.50	29.2	10	149	1.49	29.1		
11	348	2.00	39.0	11	287	1.36	26.5		
12	200	1.80	35.1	12	161	1.47	28.7		
13	160	1.60	31.2	13	179	1.28	25.0		
14	160	1.70	33.2	14	153	1.47	28.7		
15	100	2.00	39.0	15	247	1.68	32.8		
16	175	1.83	35.7	16	368	1.58	30.8		
17	249	2.20	42.8	17	264	1.65	32.2		
18	216	1.85	36.1	18	136	1.38	26.9		
19	206	1.30	25.4	19	266	1.56	30.4		
20	266	1.03	20.1	20	262	1.37	26.7		
21	102	1.66	32.4	Average	227	1.49	29.1		
22	198	1.54	30.1						
23	179	1.68	32.8						
24	334	1.91	37.3						
25	254	1.65	32.5	Total average		213	1.59	31.1	
Average	199	1.70	33.2						

<sup>1</sup> The expense of this study was defrayed in part by a grant from the Horace H. Rackham and Mary A. Rackham Foundation.

\* One healthy dietitian on approximately the same diet as the others had a consistently higher plasma carotene. Because of the great difference, we felt justified in omitting it from the normal series.

determinations were made was not necessarily fasting blood, since Kimble (4) has shown that ordinary meals do not increase the concentration of either vitamin A or carotene within two to six hours after ingestion. The range was found to be 20.0 to 43.0 micrograms of vitamin A per 100 cc. and 100 to 368 micrograms of carotene per 100 cc., as shown in Table I. The vitamin A concentrations in this group were close to, though somewhat higher, than those obtained in rats that were shown to have adequate stores in their liver. This seems to indicate that the normal subjects used were all in a good vitamin A nutritive state. In addition, our range is in close agreement with that found by Kimble (4) (1.2 to 2.9 L 620 units of vitamin A and 100 to 360 micrograms of carotene per 100 cc.) on a larger group of healthy controls. Our data show a sex variation, which, again, is in agreement with Kimble. The women have slightly lower vitamin A and higher carotene values than the men. The difference, however, is of doubtful significance.

Results on sixteen diabetic patients are shown in Table II. Those included in the study were chosen because they appeared to be of the juvenile type and were selected from the patients who presented themselves to the clinic for care. Except for the three from whom we obtained carotene tolerance data, we collected our samples without interfering with the clinical treatment and in only a few cases were the patients glycosuric. During the experimental period, the three patients on whom carotene tolerance tests were made were

controlled with insulin. Two of them remained aglycosuric throughout the period and the third showed a small amount of sugar in two of four daily urine specimens. The ages of all patients ranged from 13 to 41 years. Ten were under 25, the remaining six over 25. All presented a history of the typical onset of the disease—polyuria, polyphagia, polydipsia and weight loss. Twelve had ketonuria on various admissions and of the remaining four, three were admitted in diabetic coma and the fourth gave a history of such an attack. In every case the fasting blood sugar before treatment was more than 187 milligrams per cent. With the use of diets containing from 150 to 300 grams of carbohydrate, it was necessary to use insulin in every case in amounts ranging from 30 to 105 units daily. The diabetes in these sixteen patients ranged from moderate to greatest severity, as would be expected in any such group of young diabetics.

The vitamin A values for the juvenile diabetics are surprisingly constant. With the exception of one high value (56.6 mgm. per 100 cc.) in a patient with xanthoma diabeticorum, all fell within the normal range and the average is the same as it is for the normal subjects. The constancy of the vitamin A concentrations in these patients was not related to the widely varying carotene concentrations. A large number of the latter fall above the normal range, and the average is significantly higher than is the carotene average of the normals.

Carotene tolerance data with both normal and diabetic subjects were also obtained. In this way we were able to compare the concentrations of vitamin A and carotene attained in the blood and the subsequent rate of decline in the two classes of subjects after administration of a massive dose of carotene. In all cases several determinations were run during the week prior to administration of carotene to determine the average concentration in the blood for each subject. During this time the subjects were on a standard diet containing 7500 International Units of vitamin A. Eighty milligrams (130,000 International Units) of carotene in oil were given by mouth. Blood analyses were made at two, four, six, and nine hours after the administration, and daily thereafter for the ensuing five days. A summary of the results is presented in Figure 1.

TABLE II  
*Carotene and vitamin A blood values for diabetic subjects*

Subject	Carotene	Vitamin A		Condition
	micrograms per cent	L units $\times 10^3$	micrograms per cent	
1. Male	372	1.59	31.0	Controlled Carotenemia
	321	1.58	30.8	
	312	1.55	30.2	
	436	1.99	38.8	
	370	1.59	31.0	
2. Female	264	1.31	25.6	Controlled
3. Female	155	1.02	19.9	Controlled
4. Female	250	1.44	28.1	Controlled
5. Female	436	1.40	27.3	Controlled
6. Female	283	2.90	56.6	Xanthoma; high blood sugar throughout
	103	1.94	37.9	
	112	2.10	41.0	
7. Male	82	1.46	28.5	Uncontrolled Controlled
	102	2.00	39.0	
8. Male	370	1.29	25.2	Controlled Controlled
	241	1.55	30.2	
	430	1.64	32.0	
9. Male	69	1.81	35.3	Controlled
10. Male	190	1.52	29.7	Controlled
11. Male	448	1.60	31.2	+++ sugar Controlled—carotenemia
	454	1.51	29.5	
12. Male	660	1.19	23.2	Controlled—carotenemia
13. Female	229	1.10	21.4	Uncontrolled; +++ acetone
14. Male	182	1.58	30.8	Controlled
15. Male	227	1.37	26.7	Controlled
16. Male	461	1.98	32.3	Xanthoma
Average	291	1.62	31.3	

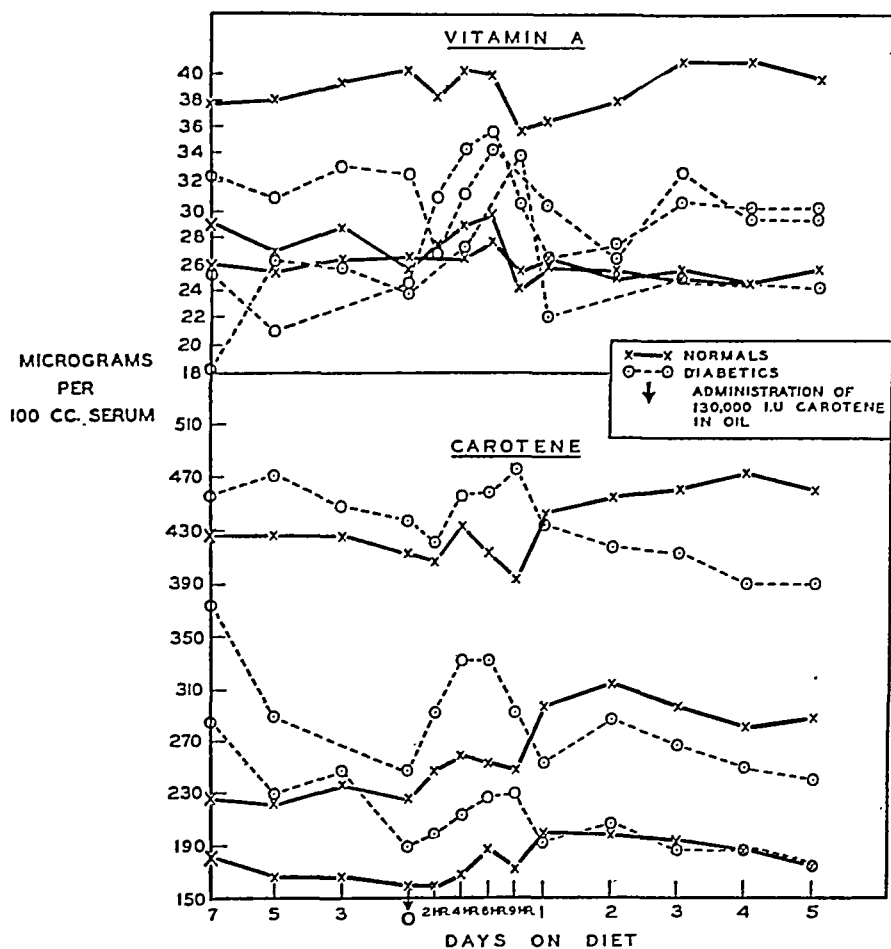


FIG. 1. BLOOD LEVELS FOR VITAMIN A AND CAROTENE BEFORE AND AFTER ADMINISTRATION OF 130,000 I.U. OF CAROTENE IN OIL TO DIABETIC AND NORMAL SUBJECTS

The concentration of carotene in the blood did not rise to as high a level as might be expected, in view of the massive dose given, in either the diabetic or the normal subjects. The stools were observed to be reddish in color, indicating that the carotene was not well absorbed from the intestine. However, the diabetics did not show a greater rise in the concentration of carotene in the blood, nor did the concentrations remain elevated longer than in the normals. The variations in the concentration of vitamin A in the blood did not alter significantly in either group of subjects throughout the experiment. This observation, plus the fact that the entire group of diabetics studied had normal concentrations of vitamin A in the blood, did not indicate that the liver of the diabetic converts carotene to vitamin A more

slowly than the normal. This study, of course, represents a small group of diabetic subjects, and the results are not statistically conclusive. Josephs (8) found that there is a relationship between vitamin A and carotene on the one hand, and total blood lipids on the other, due to the fat solubility of the former. If this is the case, the high blood fat often associated with diabetes would help to explain the high carotene values found in our diabetic group. This is an aspect of the subject that we are now investigating.

Since the variations in blood vitamin A were so small in our diabetics, we were led to the investigation of vitamin A and carotene in two normal subjects (M. M. and A. C.) placed on vitamin A low diets. In this way we wished to find out how easily and to what extent the carotene and

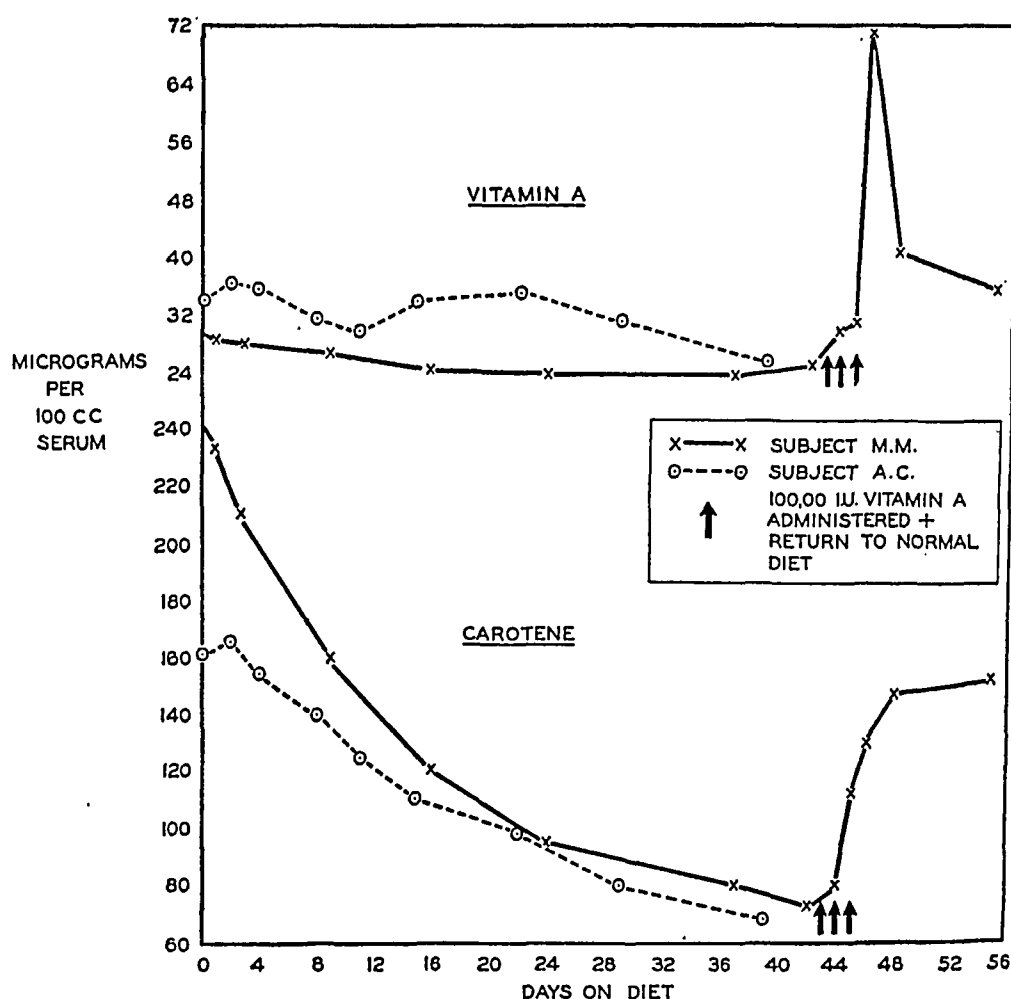


FIG. 2. BLOOD VITAMIN A AND CAROTENE DEPLETION VALUES ON TWO NORMAL SUBJECTS ON A VITAMIN A LOW DIET

vitamin A values could be lowered and whether there were concomitant physiological symptoms. The diet given was a standard vitamin A-deficient diet and was calculated to furnish 296 International Units of vitamin A per day. M. M. and A. C. remained on this diet for forty-two days and thirty-nine days, respectively. M. M. then returned to her regular diet during the period of administration of vitamin A concentrates. During this time plasma analyses for carotene and vitamin A were made at regular intervals. These values are given in Figure 2.

It is interesting to note the steady decline in carotene level until definitely subnormal concentrations were reached in both cases. The vitamin A, on the other hand, was lowered only a small amount and, at the end of the depletion period, was still well within the normal range. In the case of M. M., the vitamin A supplement (100,000 International Units as cod liver oil for four days) caused an immediate elevation in vitamin

A, reaching a super-normal concentration (70.5 mgm. per 100 cc.) on the third day, then quickly falling to within the normal range. Due to the return to a normal diet, carotene was restored to a normal value in two days. A falling carotene blood level appears to be the first sign of an impending vitamin A deficiency. Since food is the principle source of carotene for the blood, whereas the vitamin A concentrations may be maintained either from the diet or the liver stores, this would be expected. In contrast to Steininger *et al* (12), who found that the concentration of vitamin A in the blood fell markedly even during the first week on a depletion diet, in the present investigation the concentration was not lowered significantly by forty days on the special diet. If the values obtained for vitamin A in the blood are interpreted in the light of our rat studies (3), they suggest that the liver still had a plentiful store of vitamin A. Even if a drop in the blood level had indicated serious impairment to the liver stores, con-

siderable time might elapse before physiological symptoms associated with avitaminosis A would assert themselves.

Biophotometric readings according to the Jeans (7) technique were carried out during the course of depletion of M. M. and A. C., and also on various of our diabetic subjects. These data are not included in this paper because results were so variable that it was impossible to draw any conclusions from them. In addition, the reliability of biophotometric data has been widely criticized (9, 10, 11). Hecht and Mandelbaum (10), using a different technique, by which they measured both rod and cone adaptation, state that measurements of dark adaptation, when made under critically standardized conditions, may be used as an aid in the diagnosis of avitaminosis A. Steininger and Roberts (11, 12), using the Jeans technique, have concluded that, although there is a relationship between vitamin A deficiency and biophotometer readings, it is not close enough to detect subclinical deficiencies. In addition, they were unable to find any correlation between the biophotometer readings and the concentrations of vitamin A in the blood. Since it is still a controversial subject, we felt that our data would have little significance.

It seems evident that the vitamin A plasma concentration is altered only in extreme cases of vitamin deficiency. The depletion period for the two subjects just discussed was not long enough to reach this critically low level of vitamin A. The diabetic group showed that the disease did not at all affect normal vitamin A concentrations in the blood. However, there is some disturbance in carotene metabolism in diabetes, since many of our patients had clinical carotenemia, as evidenced by yellow pigment deposits, especially in the palms of the hands. Our evidence leads us to conclude that it is not necessarily an impairment of the mechanism that converts carotene to vitamin A.

#### SUMMARY

1. The normal blood plasma concentrations for carotene and vitamin A, determined according to the method of Kimble, were found to be 100 to 368 micrograms per 100 cc. for carotene and 20.0 to 43.0 micrograms per 100 cc. for vitamin A.

2. Blood plasma concentrations were determined in patients with diabetes mellitus. The

average carotene concentration for the diabetic group was significantly higher than for the normal group, while the vitamin A concentrations were the same.

3. Carotene tolerance curves were run on three normal and on three diabetic subjects. There was no delay in the fall of the carotene concentrations attained in the diabetic subjects after the administration of 80 milligrams (130,000 International Units) of carotene. This may be an indication that the carotenemia observed in diabetics is not due to the failure of conversion of carotene to vitamin A.

4. Two normal subjects were depleted for forty-two and thirty-nine days. One was given vitamin A concentrate. During this time records were kept of the blood plasma concentration for carotene and vitamin A. The carotene fell to subnormal levels but the vitamin A was not appreciably changed.

The authors wish to thank Doctor Arthur C. Curtis for his many helpful suggestions and criticisms throughout this study.

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METABOLISM, TOXICITY AND MANNER OF ACTION OF GOLD COMPOUNDS  
USED IN THE TREATMENT OF ARTHRITIS. I. HUMAN PLASMA AND  
SYNOVIAL FLUID CONCENTRATION AND URINARY EXCRETION  
OF GOLD DURING AND FOLLOWING TREATMENT WITH  
GOLD SODIUM THIOMALATE, GOLD SODIUM THIO-  
SULFATE, AND COLLOIDAL GOLD SULFIDE

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There has been increasing interest in the use of gold salts in the treatment of arthritis since the favorable report of Forestier (1) in 1929. Numerous reports from various parts of the world emphasize the value of this form of treatment, especially for rheumatoid arthritis; in many instances it is stated that gold therapy has been found more beneficial than any other treatment. In the United States this treatment has become popular only in the past three years, stimulated largely by the endorsement of Key (2).

In 1938 we began a critical clinical study of this form of therapy in cases of typical rheumatoid arthritis, using gold sodium thiomalate (myochry-sine) and gold sodium thiosulfate. At the end of a year's trial, our experience led us to the following temporary conclusions: (1) gold therapy is definitely beneficial to some but not all patients with rheumatoid arthritis during the active inflammatory stage of the disease and (2) the treatment frequently produces toxic reactions, many of which may be serious. We were convinced of the potential value of this form of therapy, and equally convinced that this method of treatment was quite unsatisfactory in its existent state.

Treatment of arthritis with gold salts originated and to date largely remains on an empirical basis. We have been unable to discover any scientific basis (1) for the selection of the various gold salts commonly used, (2) for the popularity of the parenteral route of administration, (3) for determining the size of the individual doses and the total amount of the drug which will be given, and (4) for deciding upon the interval between

doses and courses of treatment. Clinical experience has led to certain preferences, many of which are conflicting. The method of action of gold salts to produce benefit or toxic reactions is not known. The literature contains very little information concerning the metabolism of gold or the fate of this element when administered in the treatment of arthritis. Methods employed in the previous studies of this subject are for the most part non-specific and lack sufficient sensitivity to give reliable results.

For these reasons, we began an intensive study of this problem with the belief that information could be obtained on the basis of which the value and limitations of gold therapy might be clearly defined; if the value of gold therapy was upheld, it was hoped that the administration of preparations of gold now available, or others that might be developed, could be put on a scientific basis so that by adequate laboratory control maximum benefit would be accomplished and toxic reactions would be avoided entirely or minimized.

We adopted the *premise* that the effects of gold salts are due to gold contained in the molecule. Our purpose was to determine the fate of gold administered in different ways in the treatment of patients with rheumatoid arthritis. In this first report results obtained with the use of gold sodium thiomalate,<sup>2</sup> gold sodium thiosulfate,<sup>3</sup> and colloidal gold sulfide<sup>4</sup> will be presented. The first two preparations, which are solutions of crystalline gold salts, were investigated because they

<sup>2</sup> *Myochry-sine*, marketed by Merck & Co., Rahway, New Jersey.

<sup>3</sup> Supplied for this study by G. D. Searle & Company, Chicago, Illinois.

<sup>4</sup> *Aurol-Sulfide*, supplied for this study by Hille Laboratories, Chicago, Illinois.

<sup>1</sup> The Rackham Arthritis Research Unit is supported by the Horace H. Rackham School of Graduate Studies of the University of Michigan.



are used more extensively than other gold compounds in this country; colloidal gold sulfide was studied in order to compare the behavior of a colloidal suspension of a gold salt with the solutions of crystalline gold compounds, and also because it could be administered orally, intravenously or intramuscularly.

#### METHODS

Patients who had typical rheumatoid arthritis with active joint synovitis were studied during treatment and for many months following treatment. In most instances gold sodium thiomalate, gold sodium thiosulfate and colloidal gold sulfide were injected *intramuscularly*; in some cases colloidal gold sulfide was administered orally entirely, in other cases it was given orally and also intramuscularly. Injections were made weekly except in rare instances when they were at five-day intervals. Salicylates and, in some cases, small doses of vitamins C and D and hypnotics were the only other drugs used during these investigations.

In some patients extensive, continuous metabolism studies were conducted similar to those commonly employed in the study of calcium and phosphorus. During the period of administration of increasing doses of the drug, and for two or three weeks after a fixed weekly dose was given, twenty-four hourly collections of urine were analyzed for gold by the method of Block and Buchanan (3). Creatinine determinations were made by the method of Folin (4) to check the complete collection of urine. The content of gold was determined in venous blood plasma obtained at the same time in the morning, usually daily. (Fecal gold was not determined during the investigation reported in this communication.)

In many other patients, twenty-four hourly collections of urine and samples of venous blood were analyzed for gold at intervals during and after treatment with gold salts. Gold analyses were made of knee joint fluid and blood obtained simultaneously from some patients. Often plasma and urine gold values were determined previous to administration, and at one, two, four, six and twenty-four hours after giving a gold salt (a method similar to a glucose tolerance test) in order to learn the speed of absorption of gold from intramuscular depots or the intestinal tract, and the resultant urinary excretion of gold.

The gold content of the compounds used during this study was frequently determined.

Since the amount of gold employed is small, the analytical method must be extremely sensitive, specific, and accurate. Among the available methods for gold analysis, we found none which was sufficiently specific and sensitive to be employed satisfactorily in the analysis of biological fluids and tissue. A photoelectric colorimetric micromethod was devised by Block and Buchanan (3) specifically for this work; this procedure has been highly satisfactory and has been employed in all of our investigations.

#### RESULTS

Samples of blood and urine obtained from patients who were not treated with gold compounds, and from normal control subjects, never contained gold by our method of analysis. Since all the gold in blood was found to be present in the plasma, all analyses were made on plasma and are reported in terms of plasma concentration.

TABLE I

*The gold present in plasma and synovial fluid obtained simultaneously from patients treated with gold sodium thiomalate and colloidal gold sulfide intramuscularly*

Patient	Preparation	Weekly intake of gold	Gold in plasma	Gold in synovial fluid
		<i>mgm.</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>
T. W.	Gold sodium thiomalate intramuscularly (Myochrysine)	12.5	0.03 0.33	0 0
K. N.	Gold sodium thiomalate intramuscularly (Myochrysine)	25	0.42*	0.43*
F. M.	Gold sodium thiomalate intramuscularly (Myochrysine)	25	0.16 0.59*	0.11 0.15*
L. W.	Gold sodium thiomalate intramuscularly (Myochrysine)	50	0.25	0.24
O. J.	Gold sodium thiomalate intramuscularly (Myochrysine)	50	0.07†	0†
C. K.	Colloidal gold sulfide intramuscularly	15	0.07 0.19* 0 0.20*	0 0* 0 0.21*
S. C.	Colloidal gold sulfide intramuscularly	43	0.18 0.08	0.11 0.03
F. S.	Colloidal gold sulfide intramuscularly	60	0.04	0.05

\* Four hours after injection.

† Ten months after last injection.

Results obtained in subject M. B. (Figure 1) are characteristic of those obtained in three other persons treated with gold sodium thiomalate and given increasing amounts supplying from 10 to 50 *mgm. of gold*, until 500 *mgm. of gold* had been administered. It should be particularly noted that, throughout this study, dosage is referred to in terms of the *gold* and *not the quantity of the gold salt*; in this way the gold administered in the different drugs can be readily compared.<sup>5</sup>

Gold was found in the blood in increasing amounts as the amount of gold injected increased. Between injections, the daily plasma values fluctuated somewhat but, in general, after each injection

<sup>5</sup> The dose of the drug can be readily computed; gold sodium thiomalate is 50 per cent gold by weight, gold sodium thiosulfate is 37 per cent gold, and colloidal gold sulfide is 87 per cent gold.

## GOLD IN PLASMA AND URINE

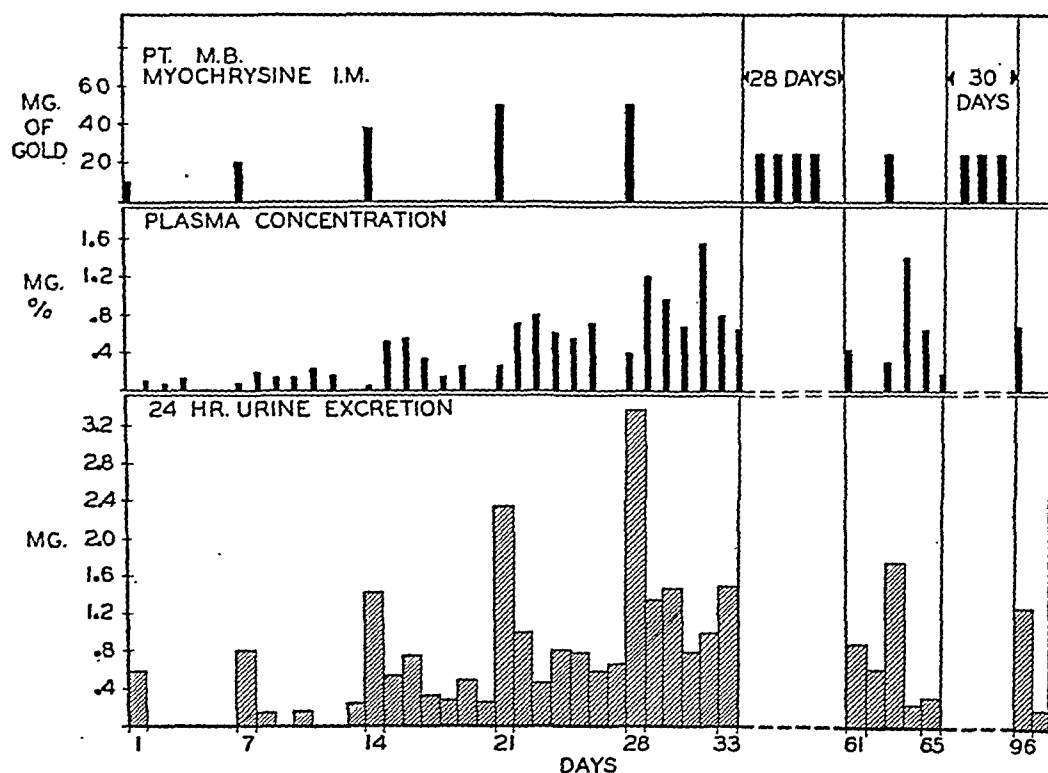


FIG. 1. PLASMA CONCENTRATION AND THE EXCRETION OF GOLD IN THE URINE OF A PATIENT WITH RHEUMATOID ARTHRITIS TREATED WITH GOLD SODIUM THIOMALATE (MYOCHRSINE) INJECTED INTRAMUSCULARLY AT WEEKLY INTERVALS

Increasing doses were employed until two doses containing 50 mgm. of gold were given; thereafter, 25 mgm. of gold were injected.

tion the plasma gold concentration increased and either remained at this higher level or decreased slightly until the next injection was given. The plasma concentration after the first injection of 50 mgm. of gold (between the twenty-first and twenty-eighth days) averaged about 0.8 mgm. per cent. Subsequently, after weekly injections of 25 mgm. of gold, the average plasma content was less; it did not increase as more gold was administered. Similarly, the excretion of gold in the urine increased as larger amounts of gold were injected; the increased excretion, however, was *not directly proportional* to the increased administration of gold. Although fecal analyses were not done in the study of this patient, in others we have found that the urine is the chief route of excretion of gold administered in this compound. Thus it is seen that gold is being retained in the

body in increasing amounts as the size of the injection is increased. This apparent retention of gold is of important magnitude; for instance, after 50 mgm. of gold were injected on the twenty-first day, an average of only 1 mgm. was excreted daily during the following week, so that only 7 mgm. of the 50 mgm. of gold injected (14 per cent) were eliminated in the urine before another 50 mgm. were injected on the twenty-eighth day. Thus one needs to account for the remaining 86 per cent of gold. In this connection, the finding of significant plasma concentrations and continued urinary excretion of gold long after its administration ceased, as will be demonstrated later (Figures 7 and 8), is extremely interesting. The excretion of gold did not increase after the amount of gold injected was no longer increased; the excretion was definitely less after the weekly dose of

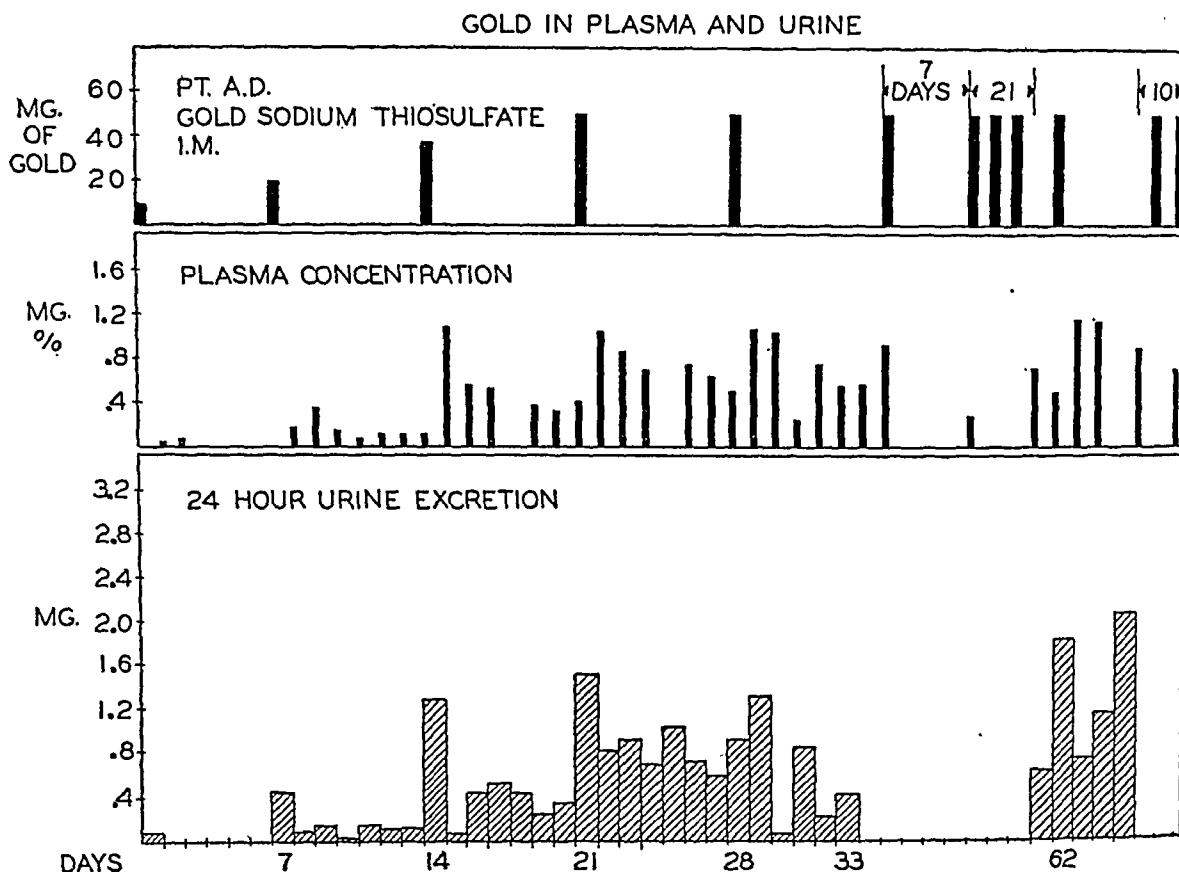


FIG. 2. PLASMA CONCENTRATION AND EXCRETION OF GOLD IN THE URINE OF A PATIENT WITH RHEUMATOID ARTHRITIS TREATED WITH GOLD SODIUM THIOSULFATE, INJECTED INTRAMUSCULARLY AT WEEKLY INTERVALS

Increasing doses were employed until the dose contained 50 mgm. of gold, at which level it remained throughout the remainder of the study.

gold was reduced to 25 mgm. (Compare sixty-first to sixty-fifth days with twenty-first to thirty-third days.) Without exception the excretion of gold was much greater on the day of injection than on other days. These high urine values on the day of injection were quite likely the result of the quick rise in plasma concentration after injection (Figure 4); however, the excretion decreases more quickly and to a greater degree than does the plasma gold content.

Findings in the case of A. D. (Figure 2) are characteristic of the results obtained when gold sodium thiosulfate was given in precisely the same way and in doses providing equivalent amounts of gold, except that after the dose was increased to an amount containing 50 mgm. of gold it was kept at this level thereafter. In general, the plasma and urine gold values are strikingly similar to those obtained with gold sodium thiomalate. The urinary excretion of gold on the day of injection was greater than on subsequent days, but usu-

ally less than occurred when gold sodium thiomalate was administered. With this drug, as with gold sodium thiomalate, the plasma and urine values did not increase after the weekly dose was no longer increased, even though gold was being retained throughout the course of treatment.

Results obtained with colloidal gold sulfide were exceedingly variable. Findings in M. R., the first patient studied extensively while this drug was employed, appear in Figure 3. Colloidal gold sulfide was administered daily in amounts providing 182 mgm. of gold per week, which was more than was given either as gold sodium thiomalate or as gold sodium thiosulfate. This patient absorbed gold both from the intestinal tract and from muscle depots when it was administered in this colloidal form. The concentration of gold in blood obtained usually every day (before treatment was given that day) varied somewhat from day to day; the average was slightly higher when it was given orally three times a day than when

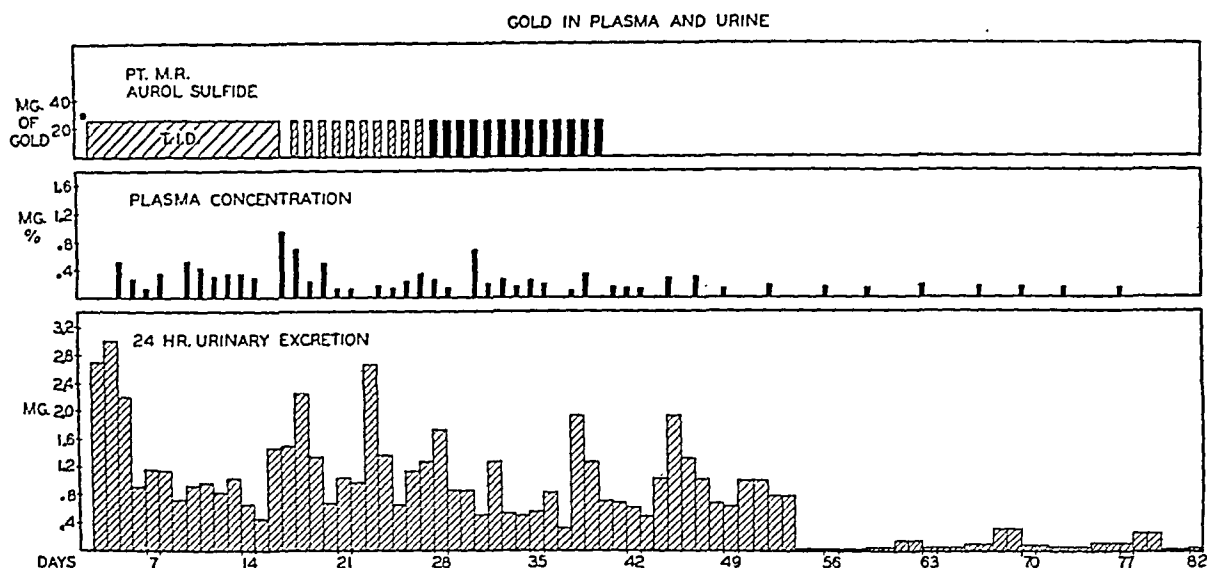


FIG. 3. PLASMA AND URINE VALUES IN A PATIENT WITH RHEUMATOID ARTHRITIS TREATED WITH COLLOIDAL GOLD SULFIDE

During the first sixteen days 8.7 mgm. of gold were given orally three times daily; from the seventeenth through the twenty-seventh day the drug was given orally in a single morning dose equivalent to the total daily dose during the previous period; beginning with the twenty-eighth day this same daily dose was injected intramuscularly.

it was given in a single morning dose. There was no difference in plasma concentration whether the drug was ingested or injected. Except for the first few days when the urine content was unexplainably higher, the average excretion of gold in urine was about 1 mgm. daily. Thus with a larger intake of gold given as colloidal gold sulfide, the gold concentration of the plasma was significantly lower and the excretion of gold was about the same as when only 50 mgm. of gold were injected intramuscularly as a true aqueous solution of either gold sodium thiomalate or gold sodium thiosulfate. A significant blood and urine content of gold existed for six weeks after the last administration of gold sulfide. The studies ceased at this time; how much longer gold would have been found in the blood and urine of this patient we do not know.

It should be emphasized that the findings in this patient (M. R.) are *not characteristic* of others treated with colloidal gold sulfide, but rather the exception, in that both the plasma and urine content of gold was much higher in this patient than in most others studied. This will be emphasized again when average values obtained with different gold compounds are contrasted (Table II). The findings in this case (M. R.) show that some

persons do absorb gold from the intestinal tract and from muscle depots when colloidal gold sulfide is administered. In some patients similarly treated no gold was found in the blood or urine; in most subjects studied there was considerable variation in the values, but with rare exceptions the values in both blood and urine were much lower than were found in M. R.

TABLE II

*The average plasma and urine gold values obtained from analyses of many specimens from many different patients during the latter part of treatment with gold sodium thiomalate, gold sodium thiosulfate and colloidal gold sulfide.*

The values for the combined oral and intramuscular administration of gold sulfide are averages of analyses in only one patient.

Preparation	Route of administration	Average weekly intake of gold	Gold in plasma	Gold in urine (twenty-four hourly excretion)
		mgm.	mgm. per cent	mgm.
Gold sodium thiomalate (Myochrysine)	Intramuscularly	50	0.44	0.75
		25	0.33	0.63
		12.5	0.26	0.23
Gold sodium thiosulfate	Intramuscularly	37.4	0.45	0.98
		19	0.28	0.43
Colloidal gold sulfide	Orally	60	0.07	0.21
	Orally and intramuscularly	140	0.01	0
	Intramuscularly	43	0.18	0.18

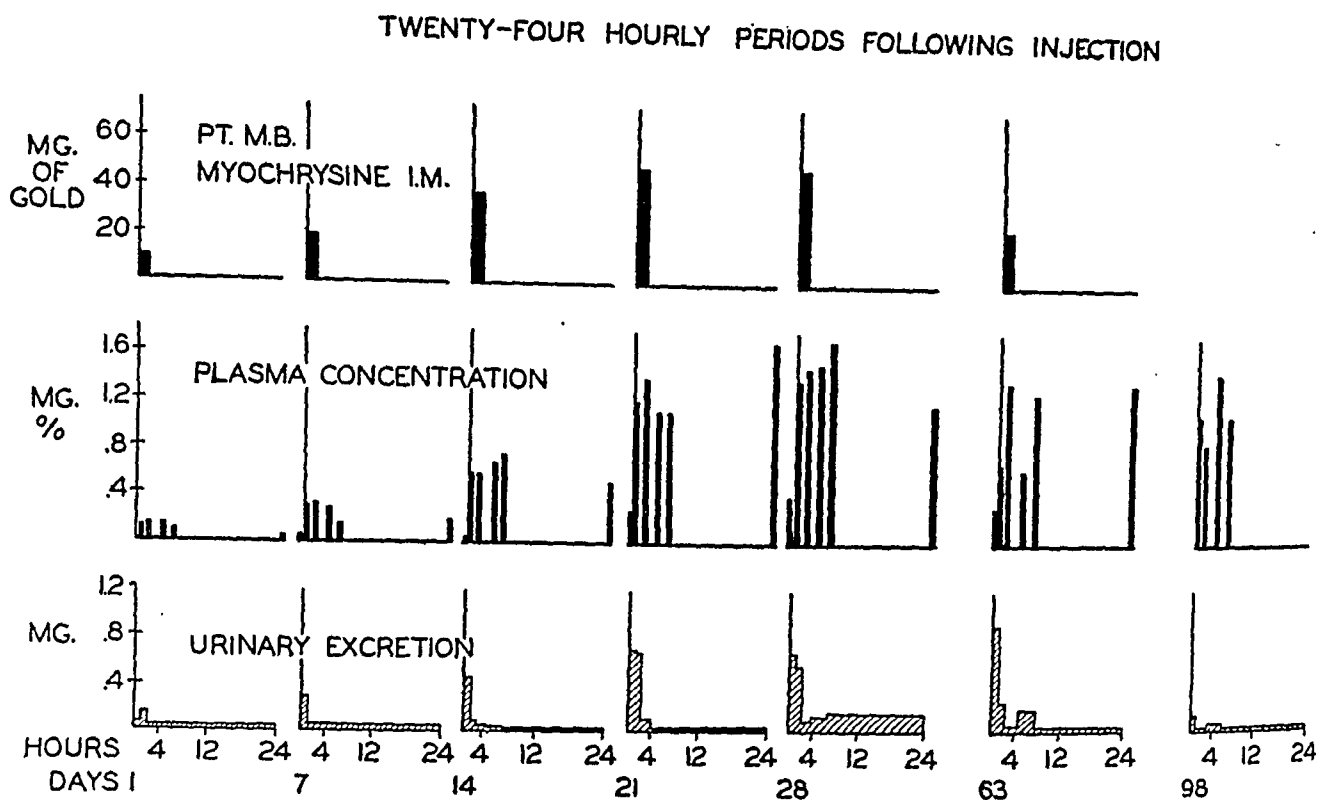


FIG. 4. PLASMA CONTENT OF GOLD BEFORE THE INTRAMUSCULAR INJECTION OF GOLD SODIUM THIOMALATE AND ONE, TWO, FOUR, SIX AND TWENTY-FOUR HOURS AFTER; AND GOLD CONTENT OF THE URINE FORMED BETWEEN THE TIME OF THE BLOOD ANALYSES

The vertical lines extending through the graph indicate the time of injection. The plasma values just preceding each line represent the blood gold concentrations just before each injection. Urine values for periods longer than one hour are average hourly values determined from urine accumulating between times of plasma determinations. Only the days of injection are shown; the intervening six-day periods are indicated by interruptions in the base lines. On the ninety-eighth day no gold salt was injected but the plasma and urine were analyzed as on previous injection days.

Information concerning the absorption into the blood stream, disappearance from the blood, and the urinary excretion of gold was obtained by analyses of plasma and urine during the first few hours after administration of a gold salt. In Figures 4, 5, and 6 are the results of such studies made in the same patients whose daily findings appear in Figures 1, 2 and 3. In every instance, one hour after the injection of gold sodium thiomalate (Figure 4) the plasma contained a much greater amount of gold than was present just before injection, and the plasma content was higher as the amount of gold injected was increased. Subsequent analyses during the twenty-four-hour period showed plasma values essentially the same as obtained one hour after injection. Thus there was no *quick* fall in plasma gold content after the intramuscular injection of gold sodium thiomalate. The urine formed during the first hour after injection usually contained more

gold than in subsequent hours. There was little correlation between urine and plasma values; for example, on the twenty-first and also on the twenty-eighth day the urinary excretion of gold decreased during the twenty-four-hour period after injection, while the plasma values remained almost constant.

Results obtained with gold sodium thiosulfate (Figure 5) were almost identical to those found with gold sodium thiomalate, except that the urine values were smaller and the excretion was slower.

The plasma contained different amounts of gold at different hours in a twenty-four-hour period when colloidal gold sulfide was given orally in equal doses three times daily (Figure 6). This may have been the result of an inconstant rate of absorption from the intestinal tract or an inconstant rate of removal from the blood. On the days when gold sulfide was given in a single morning dose, the plasma content was more con-

## TWENTY-FOUR HOURLY PERIODS FOLLOWING INJECTION

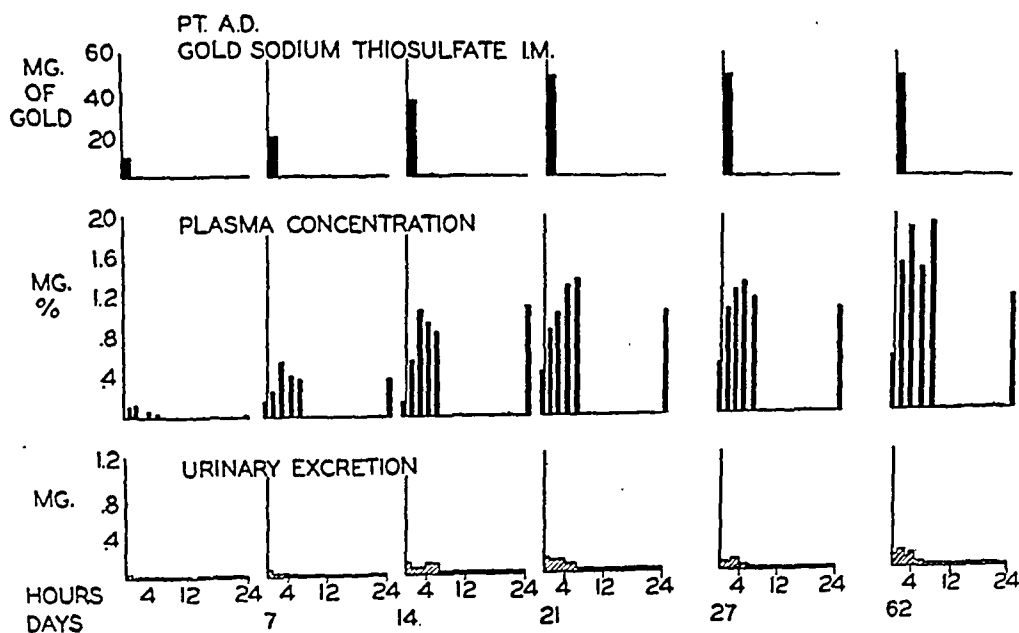


FIG. 5. PLASMA CONTENT OF GOLD BEFORE THE INTRAMUSCULAR INJECTION OF GOLD SODIUM THIOSULFATE, AND ONE, TWO, FOUR, SIX AND TWENTY-FOUR HOURS AFTER; AND THE GOLD CONTENT OF THE URINE FORMED BETWEEN THE TIME OF BLOOD ANALYSES

The injections are indicated by the vertical lines extending through the graph. Plasma values shown just before these lines represent the blood gold concentration just before the injections. Urine values for periods longer than one hour are average hourly values.

stant; there was no significant difference whether the salt was given orally or intramuscularly. Excretion of gold was greater during the first two hours after administration than subsequently. Again it must be emphasized that the findings in this case are *not characteristic* of others treated with this preparation of gold, most of whom had much lower plasma and urine values.

Comparison of plasma and synovial fluid gold content of specimens obtained simultaneously can be made from the data in Table I. In some patients plasma and synovial fluid values were almost identical, in others the joint fluid contained less gold than did the plasma, in still others no gold was found in the synovial fluid. Gold quickly diffused into the joint space in some instances, as results indicate in K. N. and the last value obtained in C. K. (In each case analyses were made four hours after an injection of gold.) This was not true in other cases. The presence of a small amount of gold in plasma and none in the joint

fluid of O. J. ten months after the administration of gold ceased indicates that gold did not remain in synovial fluid longer than in the plasma. Synovial fluid concentration was never significantly higher than the plasma concentration. Differences in synovial fluid gold content, as compared to plasma content, observed in different patients may very well be due to structural variation and permeability in synovium and joint capsule as a result of differences in degree of inflammation and duration of the disease.

The three gold salts employed in this investigation can be compared further by studying the *average* values of many analyses of blood and urine obtained to date from many different patients during the latter part of a course of treatment (Table II).<sup>6</sup> Plasma and urine values were higher when larger amounts of gold were admin-

<sup>6</sup> As more data are accumulated with subsequent study, these averages may change.

# TWENTY-FOUR HOURLY PERIODS FOLLOWING ADMINISTRATION

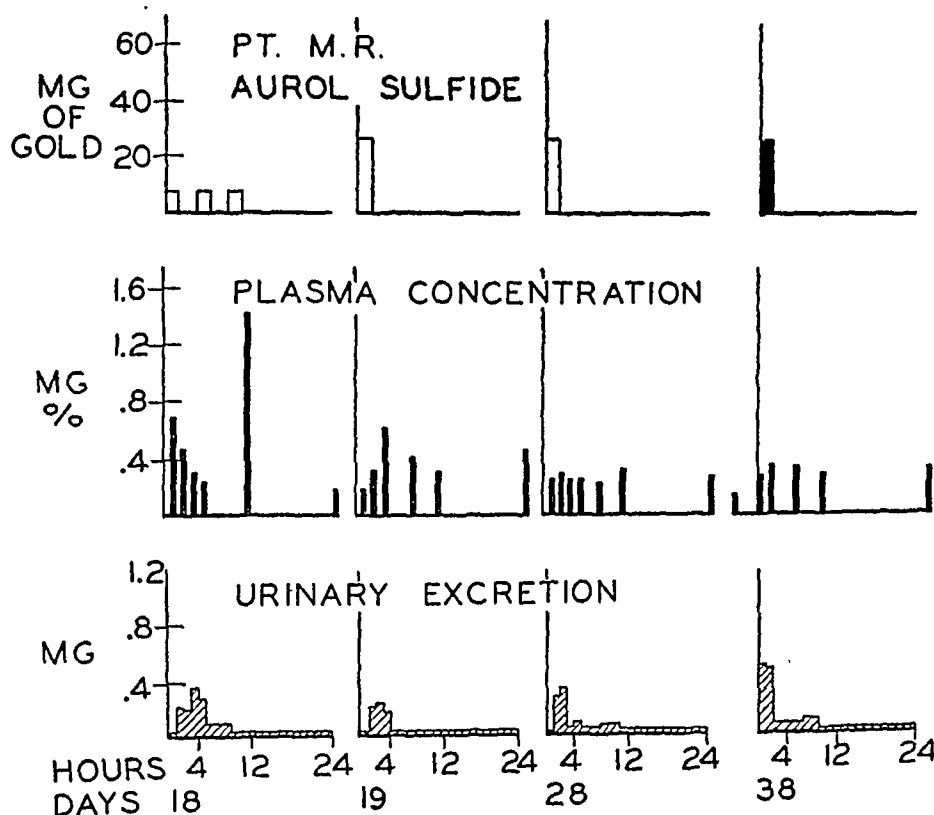


FIG. 6. PLASMA CONCENTRATION OF GOLD DETERMINED AT SHORT INTERVALS AFTER THE ORAL AND INTRAMUSCULAR ADMINISTRATION OF COLLOIDAL GOLD SULFIDE

On the eighteenth day three equal doses, each supplying 8.7 mgm. of gold, were given orally; on the nineteenth and twenty-eighth days 26.1 mgm. of gold were given in a single morning dose orally, and on the thirty-eighth day, the same dose was injected intramuscularly. Urine values for periods longer than one hour are hourly averages.

istered either as gold sodium thiomalate or gold sodium thiosulfate, but they were *not proportional* to the weekly intake of gold. Average plasma and urine values were comparable when similar amounts of gold were given as the sodium thiomalate or sodium thiosulfate salt (both crystalline). These data also show the small amount of gold that was eliminated in the urine *during* the period of administration of these salts. Very different results were obtained with colloidal gold sulfide; the average values in plasma and urine were very small, even though much more gold was given than when the crystalline preparations were used. The route of administration of the gold sulfide was not a significant factor.

One of the most important results of this investigation was the finding of significant amounts of

gold in the blood and urine for a long time *after the administration of gold salts had ceased*. A typical example of this appears in Figure 7. This patient was treated with gold sodium thiomalate in the manner commonly employed, *i.e.*, she was given intramuscular injections of this drug beginning with an amount which contained 10 mgm. of gold and increased until 50 mgm. of gold were injected. This dose was given weekly until the patient had received over 500 mgm. of gold (1010 mgm. of the drug). Plasma contained gold each time an analysis was made throughout the first thirteen weeks after the last injection, and the urine contained gold for sixteen weeks after treatment stopped. Similar results were always obtained in the many patients studied.

The length of time gold was found in plasma

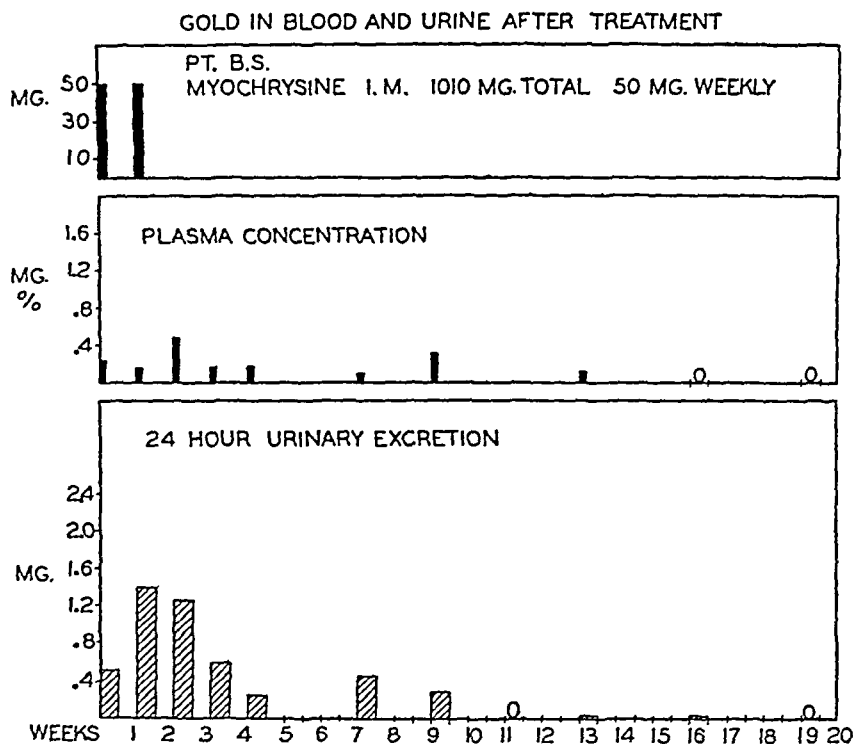


FIG. 7. SHOWING THE LONG PERIOD After Treatment with Gold Sodium Thiomalate (Myochrysine) During Which Gold Was Found in Significant Amounts in Plasma and Twenty-four Hourly Urine Collections

After initial increasing doses of the drug, 50 mgm. of gold (100 mgm. of the salt) were injected at weekly intervals until 1010 mgm. of the salt had been given. The last two injections and the corresponding plasma and urine values are shown.

is correlated with the size of the weekly doses of gold sodium thiomalate in Figure 8. In the case of those patients who received 50 mgm. of gold weekly, the earliest the urine was found to contain no gold was six months after the last injection. Some patients were excreting gold seven, nine, and ten months after treatment ended. The urine of three patients given weekly injections of 25 mgm. of gold was negative three months after the last injection. One of two patients who received 12.5 mgm. of gold weekly until 160 mgm. had been given excreted no gold in the urine one month after the last injection. Although these observations are not completed, these data indicate that the excretion of gold continues for a long time after administration of gold is stopped, and that the length of time is approximately proportional to the size of the weekly dose. It seems very significant, too, that five of the six persons who received large weekly doses of gold (Figure

8) had significant toxic reactions (lingering generalized exfoliative dermatitis, nephritis, stomatitis and gastro-intestinal difficulties). None of the eight who received 25 mgm. of gold weekly had any evident toxic reaction to gold; one of the two patients receiving the smallest dose had purpura which disappeared ten days after the drug was stopped. Hence, serious toxicity seems to be definitely related to the amount of gold injected and the speed of administration.

The results of plasma analyses were similar to the findings shown in Figure 8, indicating that gold circulates in the body for a long time after its administration is stopped.

#### DISCUSSION

The data of this report are only the first results of an investigation of the metabolism, excretion and toxicity of gold in relation to its use in the treatment of rheumatoid arthritis. Consequently,



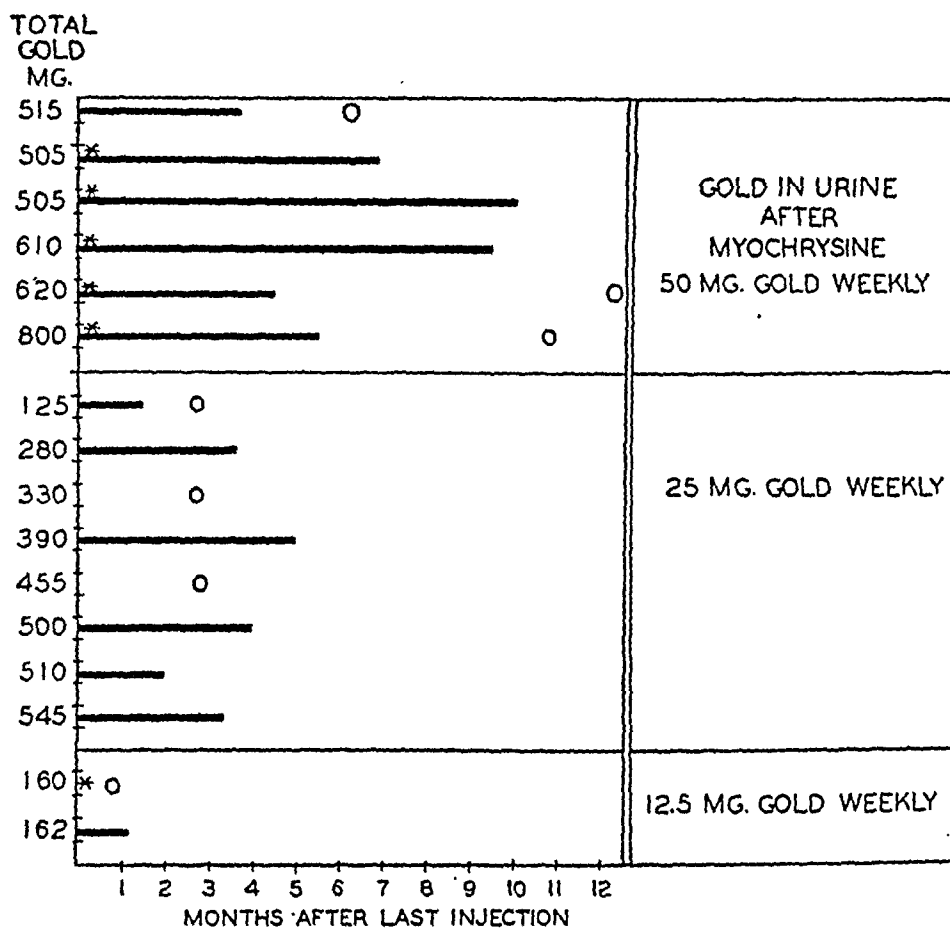


FIG. 8. SHOWING THE LENGTH OF TIME *After* TREATMENT WITH GOLD SODIUM THIOMALATE THAT GOLD WAS FOUND IN URINE, CORRELATED WITH THE SIZE OF THE WEEKLY DOSE

Each line represents a different patient and is extended to the time of the last analysis showing gold. When an analysis showed no gold, a zero is placed at the time that the analysis was made. An asterisk represents a significant toxic reaction to gold.

few conclusions can be drawn; at the same time these results indicate the nature of some interesting and valuable information which will undoubtedly be obtained as the investigation proceeds. Already certain facts are evident, and similarities of some gold medications and differences in others are clearly shown.

Gold sodium thiomalate and gold sodium thiosulfate are crystalline compounds which have similar solubilities and physical properties, and the metabolism of gold when injected intramuscularly in an aqueous solution of either of these two salts is similar.<sup>7</sup> The plasma and urine gold values are much alike for each of these salts and, although variations occur, the results are much more uni-

<sup>7</sup> Animal investigations now being conducted further emphasize the similarities in the metabolism of these two gold salts.

form than are obtained with colloidal gold sulfide. The lower values commonly found after the oral and intramuscular administration of colloidal gold sulfide may be due to slower or less complete absorption into the blood. They may, however, be the result of very quick removal of gold from the blood and its fixation in certain tissue. We are at present studying the effects of the *intravenous* injection of this colloidal gold salt. These investigations correlated with animal studies that are in progress are expected to add much information. The lower toxicity reported for this colloidal gold preparation (which we are observing) seems quite clearly to be related to the lower blood gold concentration and lower urinary excretion of gold given in this form. Similarly, if benefit from this preparation depends upon the circulation of a certain minimal amount of gold for a minimal length

of time, therapeutic failures are to be expected in those persons whose plasma gold values are very small or zero. These problems are being investigated further.

The retention of gold during treatment and the prolonged excretion of gold after the intramuscular injection of gold sodium thiomalate and gold sodium thiosulfate readily explain the slowly developing, but long-maintained apparent benefit of gold therapy commonly observed. Similarly, toxic reactions usually reflected as damage to the skin, kidneys, liver and intestinal tract, which so often appear late in the course of treatment or even follow treatment by the conventional method of injecting these salts at weekly intervals in amounts providing 50 mgm. or more of *gold*, seem, in the light of our results thus far, to be due to the increasing retention of gold until it accumulates in amounts sufficient to damage these tissues. When such large doses of gold are given at frequent intervals, significant retention of gold may result due to its *slow* excretion, so that for many weeks its damaging effects continue, thus causing serious illness which may end fatally.

The toxic reactions to gold which we have encountered to date are of at least two kinds. Most commonly, reactions have occurred late in the course of treatment; these develop slowly and persist for many weeks. These toxic reactions are manifested chiefly as generalized dermatitis which sometimes becomes exfoliative; in fewer instances, they are manifested as nephritis. Thus far we have encountered such reactions only in patients who received many large weekly doses of gold (approximately 50 mgm.). It is our opinion that such reactions result from protoplasmic poisoning effects of accumulated amounts of gold and we believe they can be eliminated, or at least shortened in duration, by avoiding large, frequent doses and thus preventing a large retention of gold. Another type of reaction, which has been observed to occur shortly after an injection of the drug (not necessarily a large dose), is manifested as apprehension, headache, flushed face and tightness in the throat, or as a purpuric dermatitis due to capillary damage. Such reactions are less common; they occur usually early in the course of treatment, and appear to be manifestations of true drug sensitivity and not due to protoplasmic, heavy metal poisoning. Prevention of such reactions

will quite naturally depend on measures unrelated to metabolic and excretory considerations.

In these studies, gold salts were given parenterally only by intramuscular injection. The blood concentration of gold will naturally depend upon the balance between the speed and duration of absorption of gold into the circulation and the rate of its removal from the blood into the tissues or for excretion. Gold is absorbed and found in the blood soon after intramuscular injection; for how long absorption from muscle depots continues and how completely, these studies do not indicate. The importance of the absorption of gold is being investigated further in humans by studying intravenously-injected gold preparations. Complete excretion studies are also being made, including daily analyses of feces for gold. Animal studies are being conducted to learn the sites of the deposition of gold in tissues.

The manner in which gold salts might produce benefit, and the site of such action, are complete mysteries to us. One naturally wonders whether gold acts in some beneficial way at the inflamed joints. In this connection, it is interesting to note that gold was not concentrated in joint fluid nor did it remain in synovial fluid after it had disappeared from the blood.

If gold might be therapeutically effective when it is present in sufficient amounts in an active form in the body, and if it acts as a poison when present in excess of a certain amount, it seems reasonable that the quantitative determination of gold in plasma or in the urine might serve to regulate dosage and indicate the method of administration which would effect dependable and safe therapy. We are therefore studying this question with great interest, but much more data than are now available are required to prove or disprove this possibility.

#### SUMMARY AND CONCLUSIONS

An investigation of the metabolism, excretion, toxicity and manner of action of gold used in the treatment of rheumatoid arthritis is being conducted. In this communication, data concerning plasma and synovial fluid concentration of gold and the excretion of gold in the urine of patients treated with gold sodium thiomalate (myochry-sine), gold sodium thiosulfate and colloidal gold sulfide are reported and their significance is dis-

cussed. Although the studies are as yet incomplete, the following tentative conclusions are warranted.

Following the intramuscular or the oral administration of these gold salts, gold absorbed into the blood stream is transported in the plasma.

Gold sodium thiomalate and gold sodium thiosulfate injected intramuscularly in amounts supplying equivalent amounts of *gold* result in similar plasma gold concentrations and urinary excretion of gold; and the plasma and urine gold values vary with the size of the weekly dose but are not directly proportional to it. When colloidal gold sulfide is administered either orally or intramuscularly, the plasma and urine values vary greatly in different patients; some persons absorb gold and have plasma and urinary values comparable to those obtained with the other salts studied, but most patients have very low plasma gold concentrations and excrete little gold in the urine, even though colloidal gold sulfide is given in amounts providing much more gold than is supplied in the crystalline salts of gold studied. In some persons no gold was found in plasma or urine after colloidal gold sulfide was given in large amounts.

Gold is eliminated in the urine *very* slowly. Blood and urine contain gold in significant amounts for a *long* time *after* administration of gold; the length of time corresponds approximately to the size of the weekly dose of gold.

Toxic reactions were much more frequent in patients receiving larger weekly injections of gold

sodium thiomalate or gold sodium thiosulfate. Considering this fact in the light of the metabolism data, it would appear that many toxic reactions result from retention of gold in amounts sufficient to poison the patient and that such toxicity can be prevented by giving smaller doses of gold. Our experience to date indicates that therapeutic results are quite as good with smaller doses of these gold salts as with the conventional larger doses.

Gold is not concentrated in synovial fluid; it is present in joint fluid in an amount equivalent to or less than exists in plasma.

It is hoped that with the further pursuit of these investigations gold therapy may be placed on a sound scientific basis and that, if its value is upheld, laboratory controlled methods of administration may be developed so that this therapy will become dependable and safe.

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# THE LIVER LIPIDS AND THEIR DISTRIBUTION IN DISEASE. AN ANALYSIS OF 60 HUMAN LIVERS<sup>1</sup>

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The lipid distribution in normal human livers and in cases of cirrhosis and fatty infiltration of the liver was reported in a previous study (1). As a corollary to these observations, the livers of patients dying from other diseases were analyzed for their lipid content and distribution. The question as to whether diseases other than those directly affecting the liver may alter the lipid content and its distribution in the liver is of interest because the functions of the liver are so varied that it tends to be involved in almost any profound disturbance in the body.

The clinical methods available for determining the function of the liver in relation to fat are limited. At present, the most satisfactory index of a lipid disturbance is the ratio of free to total cholesterol in the plasma. This is altered in cirrhosis, fatty infiltration of the liver, and in parenchymatous liver disease (2), but this ratio does not give information as to the total lipid content of the liver, or the other lipid fractions.

The determinations that we are reporting were made to ascertain the changes that might occur in the liver lipids in diseases other than those directly affecting the liver. These values are compared to the normal liver lipid values obtained by analysis from a group of 25 normal subjects reported in the previous study (1). The average total liver lipid for this group of normals was 4.98 grams per 100 grams of wet liver. The range was from 2.42 to 8.41 grams per cent. The average fatty acids were 3.50 grams per cent, the average phospholipids 2.08 grams per cent, and the average neutral fat 2.26 grams per cent; the average total cholesterol was 283 mgm. per cent and the average free cholesterol 204 mgm. per cent.

Liver samples were obtained in almost all of the cases within 24 hours after death. As re-

ported previously (1), the fraction which is most subject to change as a result of standing is the phospholipid fraction, which tends to decrease after about 5 hours. There seems to be no further decrease after 24 hours. Lipids and their fractions were done by the methods previously described from this laboratory (3).

Samples of liver were obtained from patients dying of the following diseases: (1) Acute infections, such as meningitis, pneumonia, peritonitis, and septicemia; (2) chronic infections, including tuberculosis, subacute bacterial endocarditis, syphilis and osteomyelitis; (3) malignancy; (4) metabolic diseases; and (5) cardiovascular diseases. The diseases of 4 of the patients in the metabolic group were complicated by an acute infection (Numbers 3, 7, 54, 88), and 2 (Numbers 10 and 92) cases in this group also had some form of heart disease. The liver values for these 6 cases are entered in both pathological groups. The acute infections were subdivided into two groups, as 5 of these patients were diagnosed pathologically as having fatty infiltration or cirrhosis of the liver.

## RESULTS

In Table I are the findings in 21 cases in whom death was due to some acute infection. Of these 21 cases, 16 had no evidence of cirrhosis or fatty infiltration of the liver. The average weight of the livers in this group was 1760 grams. The lowest weight was 1400 grams and the greatest was 2900 grams. The average total liver lipid was 4.72 grams per cent. In one liver the total lipid was 9 grams per cent, but in the majority of the group the total lipid was 5 grams per cent or less. The lowest lipid value in the group was 2.3 grams per cent. The average phospholipid was 1.89 grams per cent and the average of the neutral fat was 2.41 grams per cent. The total cholesterol averaged 269 mgm. per cent. These

<sup>1</sup> This research was aided by a grant from the Milbank Memorial Foundation.

TABLE I  
*Acute infections with and without cirrhosis or fatty infiltration of the liver*

Number case	Age	Sex	Nutrition of patient	Weight of liver grams	Total lipid grams per cent	Unsaponified grams per cent	Fatty acid grams per cent	Cholesterol		Phospholipids grams per cent	Neutral fat grams per cent	Pathological findings and diagnoses
								Free	Total			
								mgm. per cent				
ACUTE INFECTIONS WITHOUT CIRRHOSIS OF THE LIVER												
3	50	M	Fair	1770	4.96	0.62	4.46	189	259	1.65	3.48	Meningococcus meningitis, diabetes mellitus
9	65	M	Poor	1650	2.44	0.80	1.92	231	232	1.11	1.24	Streptococcus hemolyticus endocarditis, chronic passive congestion of liver
19	18	F	Good	1780	7.00	0.14	5.51		415	3.61	2.98	Staphylococcus aureus septicemia
59	32	M	Fair	1860	4.51	0.66	3.16		208	1.75	2.13	Bronchopneumonia, tetanus, normal liver
42		M	Good	1750	5.52	0.60	4.40		298	1.86	3.29	Lobar pneumonia
51	35	M		1800	5.60	0.65	4.87		210	1.46	4.10	Post-operative peritonitis
86	40	M	Good	1450	9.05	0.82	7.33					Pneumonia, normal liver
54	45	F	Obese	2900	3.39	0.54	2.26		232	1.87	1.07	Liver abscesses, enlarged liver, chronic cholecystitis
29	45	M		2050	3.15	0.72	2.17		263	1.22	1.46	Staphylococcus aureus septicemia, abscesses and areas of infarction in liver
23	50	M	Poor	2740	3.63	0.64	2.70		287	2.80	0.92	Lobar pneumonia, CPC of liver
88	67	F	Obese	1400	3.92	0.78	2.69					Bronchopneumonia, normal liver
67	50	M	Fair	1640	4.86	0.69	3.43			2.08	2.12	Generalized peritonitis, rupture of ileum
70	50	M	Good	1370	4.75	0.67	3.59			1.15	2.93	Pneumonia
93	78	M	Fair	1350	3.48	0.78	2.08					Lobar pneumonia, cardiac failure, normal liver
18	53	M	Good	1440	6.91	0.71	4.45		287	2.06	3.20	Purulent meningitis, fracture of skull, normal liver
105	71	M	Poor	1300	2.35							Acute cystitis, purulent
Average	50			1760	4.72	0.65	3.67	210	269	1.89	2.41	
ACUTE INFECTIONS WITH CIRRHOSIS OR FATTY INFILTRATION OF THE LIVER												
56	35	F	Poor	2450	11.50	0.84	10.20		337	1.99	9.28	Streptococcus meningitis, fatty and cirrhotic liver
31	38	M	Good	1650	7.68	0.74	6.72		338	1.80	5.71	Lobar pneumonia, cirrhosis of the liver
75	41	M	Poor	1910	16.10	0.39	14.90					Chronic alcoholism, lobar pneumonia, fatty liver
58	50	M	Poor	1950	28.10	1.00	25.50		389	1.97	25.30	Alcoholism, lobular pneumonia, fatty and cirrhotic liver
30	65	F	Poor	1170	14.05	0.97	13.60		238	2.53	12.50	Lobular pneumonia, fatty liver, Paget's disease
Average	43			1835	15.48	0.79	14.18		326	2.07	13.20	

figures are almost identical to the average of the values obtained in the 25 normal subjects. In the 5 cases in whom there was cirrhosis or fatty infiltration of the liver there was a definite increase in the total lipid and fatty acids. The average total liver lipid for the group was 15.48 grams per cent. The phospholipids were a trifle higher than in the other patients dying of acute infections, and the neutral fat was, of course, increased, since any increase in total lipid is due outstandingly to the increase in the neutral fat fraction. The average of the total cholesterol was increased.

These figures correspond with the liver lipid values obtained in the 25 alcoholic patients re-

ported previously, with the exception that the phospholipid fraction is somewhat higher in this group of 5 cases. Free cholesterol determinations were not done in these 5 cases but any increase in the total cholesterol is probably due to an increase in the esterified fraction.

A report of the determinations on 10 cases dying of chronic infection is made in Table II. Of these, 3 had tuberculosis, 1 had a lung abscess, 2 had endocarditis, 1 had a bronchiectasis, 1 died of malaria, another of luetic heart disease, and 1 of chronic cholecystitis. In 3 of the patients the total lipid in the liver was increased. One of these was the case with lung abscess, and the other

TABLE II  
Chronic infections

Number case	Age	Sex	Nutrition of patient	Weight of liver	Total lipid	Unsat-urated	Fatty acid	Cholesterol		Leci-thin	Neu-tral fat	Pathology of the liver and other disease processes
								Free	Total			
				grams	grams per cent	grams per cent	grams per cent	mgm. per cent		grams per cent	grams per cent	
7	57	M	Obese	1440	4.03	1.06	2.33	294	323	1.43	1.43	Bronchiectasis, cerebral arteriosclerosis
11	62	M	Poor	1370	12.05	1.32	10.80	221	312	0.87	10.70	Lung abscess
36	43	M	Poor	2000	3.24	1.20	2.29		242	1.77	1.16	Subacute bacterial endocarditis, aortic valvulitis
73	41	M	Good	1620	2.22	0.55	1.41			1.16	0.64	Malaria, liver normal
78	30	M	Good	2090	3.43	0.58	2.50					Chronic cholecystitis, emphysema, liver normal
97	27	M	Good	1550	4.44	0.74	2.96					Luetic aortitis, liver normal
82	45	M	Good	1720	15.27	0.58	14.13		265			Chronic alcoholic, pulmonary tuberculosis, fatty liver
84	38	M	Good	1850	13.30	0.59	11.91					C
95	49	M	Poor	2330	3.43	0.65	2.09			2.65	0.28	T
21	23	F	Good	1430	3.59	0.61	2.06					hepatitis
Average	41			1760	6.90	0.76	5.63	258	279	1.98	2.33	

2 were chronic alcoholics who had pulmonary tuberculosis. In the remaining cases, the total lipid was lower than the average for the normal livers, being about 3 grams per cent in most of the cases. The 2 patients who were chronic alcoholics also had pulmonary tuberculosis. As it was noted in the previous study that an increase in liver lipid was found in the livers of patients with a history of chronic alcoholism, it is probable that tuberculosis was not the cause of this increased lipid.

There were 7 patients dying with some form of malignancy (Table III). One of the cases, a patient with lymphosarcoma and metastatic nodules in the liver, had a total liver lipid of 15 grams per cent. It is interesting that most of these patients, several of whom had metastatic involvement of the liver, showed no change in the lipid content of the liver or its distribution. In 2 of the cases the total lipid values were somewhat lower than the usual normal values.

Table IV gives the observations on 12 patients with some metabolic disturbance. Three of these patients had diabetes, 8 were obese and had some other disease process, and 1 was a patient with Paget's disease of the bone. Interestingly enough, none of the diabetic patients had an increase in the total liver lipid. In 3 patients in this group the total liver lipids were elevated. Of these, 1 was the patient with Paget's disease, 1 was a patient with obesity, and 1 was a patient with coronary thrombosis and obesity. Four of the obese patients were killed in accidents, and no other pathological changes were found.

Table V presents the findings on 16 patients dying from some form of cardiovascular disease. Four of these had elevated total liver lipids; of these 4, 2 had coronary sclerosis (1 of whom in addition was an alcoholic), 1 had a coronary thrombosis, and 1 died of hypertensive heart disease. The last subject was also obese. The aver-

TABLE III  
Malignant diseases

Number case	Age	Sex	Nutrition of patient	Weight of liver	Total lipid	Unsat-urated	Fatty acid	Cholesterol		Leci-thin	Neu-tral fat	Pathology of the liver and other disease processes
								Free	Total			
				grams	grams per cent	grams per cent	grams per cent	mgm. per cent	mgm. per cent	grams per cent	grams per cent	
14	67	F	Poor	920	15.03	1.27	12.55	253	301	1.79	11.90	Lymphosarcoma, metastatic nodules in the liver, fatty infiltration, early cirrhosis
40	59	F	Fair	1800	3.29	0.62	2.00		273	2.30	0.51	Leukemia with involvement of spleen, bone marrow, lymph nodes and liver
62	59	M	Poor	1600	3.22	0.73	2.02			1.63	0.95	Carcinoma of stomach with metastases to ribs and spine
64	45	M	Poor	2250	3.22	0.75	2.02			2.27	0.15	Carcinoma of larynx with metastases to lungs and liver
68	83	M	Poor	1120	4.03	0.74	2.38			2.44	1.62	Carcinoma with metastases to lungs and lymph nodes
103	55	M	Poor	2550	2.45							Hypernephroma, no metastasis to liver
109	58	M	Good	4000	2.82		2.14		247	1.04	1.53	Carcinoma of gall bladder with metastases to liver, ascites, jaundice
Average	60				4.86	0.82	3.85	253	274	1.91	2.78	

TABLE IV  
*Disease of metabolism*

Number case	Age	Sex	Nutrition of patient	Weight of liver	Total lipid	Unsat-urated	Fatty acid	Cholesterol		Leci- thin	Neu- tral fat	Pathology of the liver and other disease processes
								Free	Total			
				grams	grams per cent	grams per cent	grams per cent	mgm. per cent	mgm. per cent	grams per cent	grams per cent	
2	56	F	Poor	1475	3.59	0.77	2.16	253	253	1.86	0.97	Diabetes mellitus, diabeto ketosis, normal liver, cholelithiasis
3	50	M	Fair	1770	4.96	0.62	4.46	189	259	1.65	3.48	Diabetes mellitus, meningococcus meningitis, pericentral atrophy of the
7	57	M	Obese	1440	4.03	1.06	2.33	294	323	1.43	1.43	Diabetes mellitus, mild obesity, lobular pneumonia, hypertensive arterial heart disease
10	50	F	Obese	1330	7.43	0.83	6.17	259	335	1.31	5.52	Arteriosclerotic heart disease, obesity, CPC of liver
22	50	M	Obese	1875	6.06	1.33	4.57	293		2.99	2.68	Obesity, normal liver
30	65	F	Poor	1170	14.05	0.97	13.60		238	2.53	12.50	Paget's disease, lobular pneumonia, fatty liver
32	54	M	Obese	1730	6.61	0.95	4.91		201	1.40	4.21	Obesity, slightly fatty liver
48	50	F	Obese	1640	10.60	0.96	8.90		326	2.01	6.85	Obesity, normal liver
52	31	M	Obese	1660	7.72	0.77	4.22		333	2.89	2.35	Obesity, normal liver
54	45	F	Obese	2900	3.39	0.54	2.26		232	1.87	1.07	Liver abscess, chronic cholecystitis, obesity
88	67	F	Obese	1400	3.92	0.78	2.69					Bronchopneumonia, obesity, normal liver
92	50	M	Obese	2490	19.63	0.46	18.71		237			Coronary thrombosis, fatty liver, obesity
Average	52			1740	7.67	0.84	6.25	258	274	1.99	4.11	

age total cholesterol for the group was above the normal average. Otherwise, the findings did not show any very striking changes.

## DISCUSSION

In 14 of the 60 cases the total liver lipids were increased. Of these 14 cases, 5 were patients dying from acute infections; of these 5, 4 were found to have some cirrhosis of the liver and 2 had previous histories of alcoholism. Three of the 14 patients died of some chronic infection, and 2 of these were alcoholics. Of the remaining 6 patients in whom the liver lipids were increased, 1 was a patient with lymphosarcoma, 1 was an obese patient with no other complication, and 4 were patients with heart disease.

In the group of patients dying of chronic infections, and in the group of patients dying of some form of malignancy, the total liver lipids were slightly lower in several instances than the average normal value.

On the basis of the analyses done, the amount and character of the fat in the liver do not seem to be influenced significantly unless the liver itself is subject to disease. Cirrhosis of the liver, the type of fatty infiltration that is seen most often in alcoholics, has more influence on the amount and distribution of the liver lipids than do other diseases. It is interesting that, in the very few diabetic cases analyzed, there was no appreciable increase in the amount of fat in the liver.

Breusch and Scalabrino (4) analyzed the liver

TABLE V  
*Cardiovascular disease*

Number case	Age	Sex	Nutrition of patient	Weight of liver	Total lipid	Unsat-urated	Fatty acid	Cholesterol		Leci- thin	Neu- tral fat	Pathology of the liver and other disease processes
								Free	Total			
				grams	grams per cent	grams per cent	grams per cent	mgm. per cent	mgm. per cent	grams per cent	grams per cent	
7	57		Obese	1440	4.03	1.06	2.33	294	323	1.43	1.43	Arteriosclerotic heart disease, cerebral arteriosclerosis
10	50		Obese	1330	7.43	0.83	6.17	259	335	1.31	5.52	CPC of liver
13	82		Good	1300	4.63	0.89	2.84		284	1.39	2.00	Arteriosclerotic heart disease, periportal cirrhosis of the liver
37	52		Good	1270	3.67	0.85	2.71		462	1.69	1.50	Coronary thrombosis, CPC of liver
6	53		Fair	1500	9.99	0.81	8.10	219	321	2.20	6.92	Coronary sclerosis, alcoholism
44	72		Good	1610	12.90	0.76	10.90		315	2.09		Coronary sclerosis, arteriosclerosis, normal liver
46	50		Good	1560	13.10	1.04	11.80		336	1.41		Coronary thrombosis
55	70		Good	1450	5.08	0.85	3.51		312	1.99	2.30	Coronary thrombosis, enlarged left ventricle
66	50		Poor	1410	4.18	0.51	2.94			2.05	1.66	
87	72		Poor	1350	3.97	0.57	3.13					
92	50		Obese	2490	19.63	0.46	18.71		237			Coronary thrombosis, enlarged and fatty liver
93	78		Fair	1350	3.48	0.78	2.08					Cardiac failure, lobar pneumonia, normal liver
97	27		Good	1550	4.44	0.74	2.96					Luetic aortitis, normal liver
98	65		Good	1600	3.27	0.65	2.05					Cardiac failure, CPC of liver and spleen
5	40		Fair	1720	3.85	0.67	2.44	236	256	2.57	0.75	Essential hypertension, pitocin poisoning, normal liver
45	55		Poor	1650	5.04	0.95	3.27		237	2.07	1.88	Ruptured cerebral aneurysm, subarachnoid hemorrhage, normal liver
Average	58			1535	6.80	0.78	5.37	252	320	1.84	4.11	

of 72 patients. Of these, 11 died of malignancy, 8 of tuberculosis, 10 of liver cirrhosis or atrophy, 4 in uremia, 6 of arteriosclerosis and hypertension, and 6 of pneumonia. The average total lipids in these groups varied from 4.72 to 7.68 grams per cent. The average total cholesterol varied from 312 to 277 mgm. per cent. In 1 of the cases of tuberculosis the total lipid was 20 grams per cent. In 1 of the cases of cirrhosis of the liver the total liver lipid was 12.5 grams per cent. The average total liver lipid for these two groups was not, however, elevated. The figures of Breusch and Scalabrino substantiate our findings. Apparently, the ability of the liver to handle fats is not easily impaired, and probably is only significantly affected when the liver itself is affected, as in cirrhosis.

#### SUMMARY

Analyses were done on 60 human livers. Of these, 16 were from patients dying of acute infections in whom no cirrhosis of the liver was found at autopsy. Five were from patients dying of acute infections in whom some cirrhosis or fatty infiltration was noted postmortem. Ten were from patients dying of chronic infections, 7 from patients dying with malignant diseases, 12 from patients dying with some disturbance of metabo-

lism, and 16 were from patients dying with some form of heart disease.

The outstanding finding was in the 5 cases of acute infections in whom cirrhosis or fatty infiltration of the liver was present. The average total liver lipid in these cases was increased well above the normal range (15.48 grams per cent), and the total cholesterol was increased. There were no striking changes in the average lipid values in the other groups studied.

We are indebted to Dr. Douglas Symmers, Director of the Department of Pathology of Bellevue Hospital, and to the Department of Hospitals and the office of the Chief Medical Examiner for the samples of liver obtained for analysis.

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# STUDIES ON BLOOD HISTAMINE IN PATIENTS WITH ALLERGY.

## II. ALTERATIONS IN THE BLOOD HISTAMINE IN PATIENTS WITH ALLERGIC DISEASE<sup>1</sup>

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It has long been thought that anaphylactic shock, as produced in the experimental animal, and the allergic reaction, as it occurs in man, may be dependent on the same basic mechanism for their production (1). Both anaphylaxis and allergy come under the heading of "hypersensitivity," which is an all-inclusive term and under which the phenomena of each may be conveniently grouped. In the search for an etiological factor, histamine has figured prominently ever since the studies of Dale and Laidlaw (2) who observed that the symptoms of histamine shock and anaphylaxis were strikingly similar. In 1929 Dale (3) postulated that histamine was released in anaphylactic shock as a result of cell injury, due to the interaction of the antibody with the antigen. This was subsequently shown to be true by Dragstedt and Gebauer-Fuelnegg (4) for the dog, and by Bartosch, Feldberg and Nagel (5) for the guinea-pig. Thus, until recently it was believed that anaphylaxis was always associated with an increase of the blood histamine, which in turn was responsible for the production of symptoms. In 1939, however, Code and Hester (6) noted that anaphylactic shock in the horse and calf was associated with a decrease in the histamine content of the blood. Rose and Weil (7) observed a similar change in the rabbit, and it was further noted that a decrease also occurred in the histamine content of some of the tissues of this species during anaphylactic shock (8). From these findings it appears that acute anaphylactic shock may be associated with a rapid increase in the blood histamine in certain species, and a marked decrease in others.

Investigations have also been carried out on the possible relation of histamine to allergic disease in

man. For the most part, the results have been neither as clear nor as consistent as those obtained in experimental anaphylaxis, and the greater part of the evidence has been of an indirect nature. Thus Lewis (9) postulated that symptoms of allergic disease might be explained by the liberation of an "H" or histamine-like substance, because of the similarity between histamine wheals and

TABLE I  
Blood histamine in cases of asthma

Case	Sex	Age	Diagnosis	Date	Blood histamine γ per 100 cc.	Symptoms
2	F	30	Chronic asthma		1.0	++
46	F	29	Chronic asthma		4.0	0
52	F	48	Chronic asthma		2.0	++++
					4.0	0
64	M	54	Asthma, bronchitis, hay fever		8.0	0
68	F	24	Asthma, bronchitis		4.0	++
71	F	27	Asthma		6.0	0
76	F	9	Asthma		3.0	++
					3.0	++++
81	M	31	Asthma		3.0	0
82	F	49	Severe asthma and bronchitis	November 11, 1939	1.0	++++
					1.0	++
				December 5, 1939	5.0	++++
				December 6, 1939	1.0	++
				December 13, 1939	4.0	+
				January 11, 1940	4.0	0
				January 12, 1940	2.0	0
83	F	10	Asthma		5.0	0
84	M	8	Asthma		6.0	0
85	F	8	Asthma and bronchitis		8.0	0
					9.0	++++
94	M	16	Asthma		3.0	0
104	F	25	Asthma		3.5	0
105	F	11	Asthma		9.0	Day after severe attack, still wheezing
106	F	43	Asthma and bronchitis		5.0	++
107	F	11	Asthma		12.0	24 hours after severe attack
108	F	14	Asthma and bronchitis		13.0	++++
124	M	16 mos.	Asthma		9.5	0
144	M	8	Asthma and bronchitis		1.5	+
145	F	38	Asthma		4.0	0
147	F	40	Asthma		3.5	0
165	F	45	Asthma and bronchitis	March 20, 1939	5.5	++++
				March 23, 1939	2.0	+
				April 1, 1939	4.3	0
				April 4, 1939	3.0	0
				May 3, 1939	3.2	0 8:30 p.m.
					3.8	0
						11:30 p.m.
229	M	19	Asthma	May 4, 1939	3.0	0
140	F	22	Asthma		9.0	++++
251	F	12	Asthma		4.0	0
74	M	53	Asthma and urticaria		19.0	++
					4.0	++
					4.0	++
275	M	34	Asthma		4.0	+++

<sup>1</sup> Presented in part at the meeting of the Society for the Study of Asthma and Allied Conditions, Atlantic City, May 1940.

<sup>2</sup> Aided by a grant from the Banting Research Foundation.

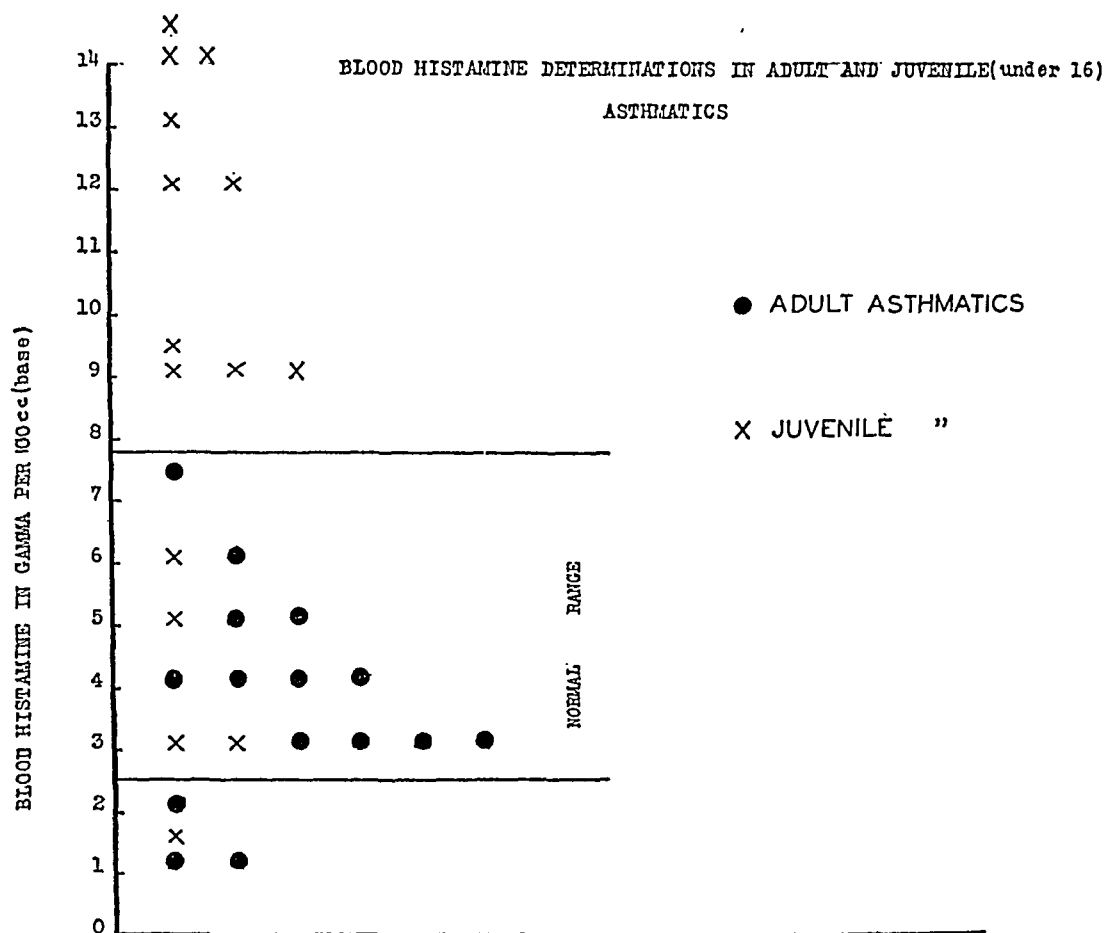


FIG. 1. THE HISTAMINE CONTENT OF THE BLOOD OF PATIENTS WITH ASTHMA CHARTED ACCORDING TO AGE GROUPS

Whereas the majority of adult asthmatics have a blood histamine level which is within normal limits (2.0 to 7.5  $\gamma$  per 100 cc.), that of many of the patients under the age of sixteen is elevated above this level.

those produced by stroking the skin of patients with dermatographia. Similarly, the liberation of free hydrochloric acid into the stomach following the production of wheals in a case of dermatographia (10) or by the immersion into ice water of the arm of a patient sensitive to cold (11) is another indirect method of demonstrating the liberation of histamine during the production of allergic phenomena.

In 1935 Barsoum and Gaddum (12) described a method for the determination of histamine in blood. Using this method or slight modifications thereof, a number of investigators have studied the blood histamine levels in patients with allergic disease. In six patients with urticaria, Cerqua (13) noted an increase of the blood histamine level during acute attacks, with a return to normal after six hours. His figures ranged from 13.0 to 30.0  $\gamma$  per 100 cc.

Observations on the histamine level of the blood in patients with asthma have been conflicting. Cerqua (13) noted a definite increase in eight cases of asthma during attacks, 18.0 to 30.0  $\gamma$  per 100 cc. During quiescent periods the blood histamine was normal, 7.0 to 10.0  $\gamma$  per 100 cc. Similar findings in a small group of cases were reported by Jacquelin (14) and Parrot (15). On the other hand, Haworth and MacDonald (16) did not find any significant alteration in the blood histamine level of sixty asthmatics. Riesser (17) was also unable to demonstrate a significant change.

In previous studies, Rose and Browne (18) observed that the blood histamine content of fifty normal persons averaged 4.0  $\gamma$  per 100 cc., with variations of from 2.0 to 7.5  $\gamma$  per 100 cc. It was also noted that the blood histamine level in any one person remained remarkably constant with little

BLOOD HISTAMINE DETERMINATIONS IN CASES OF ASTHMA  
DURING ACUTE SYMPTOMS AND IN THE QUIESCENT  
STATE

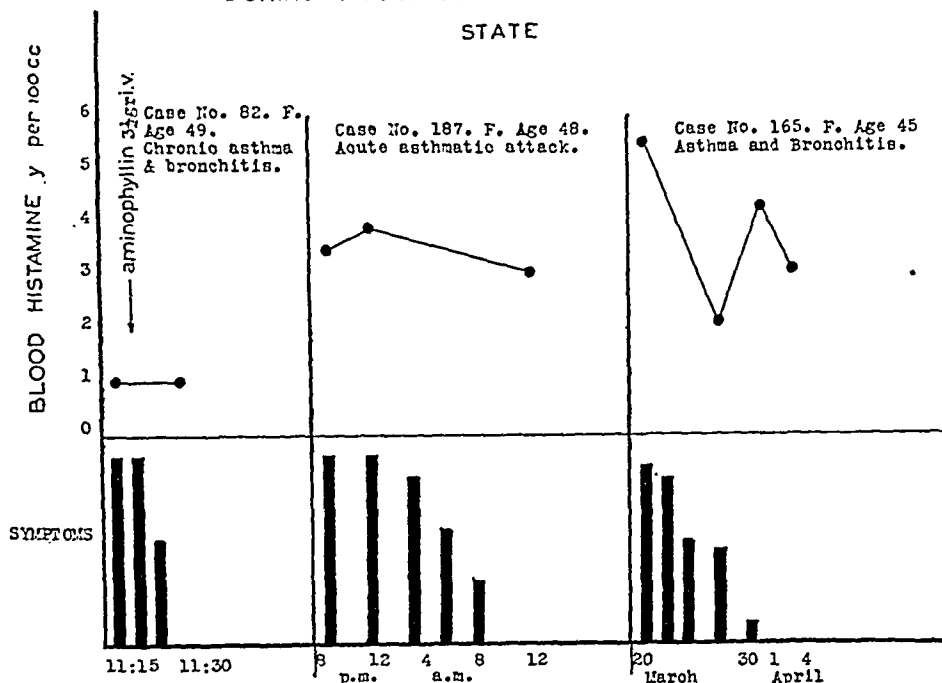


FIG. 2. THE HISTAMINE CONTENT OF THE BLOOD OF THREE ASTHMATIC PATIENTS BOTH DURING ATTACKS AND QUIESCENT PERIODS

It will be seen that, although there are variations in the blood histamine level, these are not necessarily correlated with symptoms.

or no alteration, even though examinations were made weeks or months apart. These findings agree in general with those of Code and MacDonald (19) and most other observers.

Further studies on the histamine content of the blood have been carried out in 116 patients with allergy. The results of some of these—cases of physical allergy and dermatographia—have already been reported (20). The results about to be described were obtained from observations on eighty patients with other forms of allergic disease.

#### METHODS

The blood histamine was determined by the Code (21) modification of the Barsoum and Gaddum (12) method. All assays were carried out on the guinea-pig ileum suspended in Tyrode solution at 38° C. to which atropine was added in a concentration of 1:10,000,000. This method is reasonably accurate in that small amounts of histamine may be detected to within 95 to 98 per cent when added to whole blood.

All results are expressed as gamma (gamma or  $\gamma$  = 0.001 mgm. or microgram) of histamine base per 100 cc. whole blood.

#### RESULTS

##### (a) Cases of asthma

Thirty cases of asthma, most of which were free of other allergic complications, were studied. The results are given in Table I. It will be observed that some of these patients have a blood histamine level which is below the normal (Cases 2, 82, 144), whereas others (Cases 64, 85, 105, 107, 108, 124, 239, 251 60) have a blood histamine level which is greater than normal. When these are charted according to age groups, it appears that patients of less than sixteen years of age tend to have high blood histamine levels as compared to adult asthmatics (Figure 1).

It will further be noted that the histamine content of the blood of patients with asthma tends to fluctuate greatly as compared to that of normal

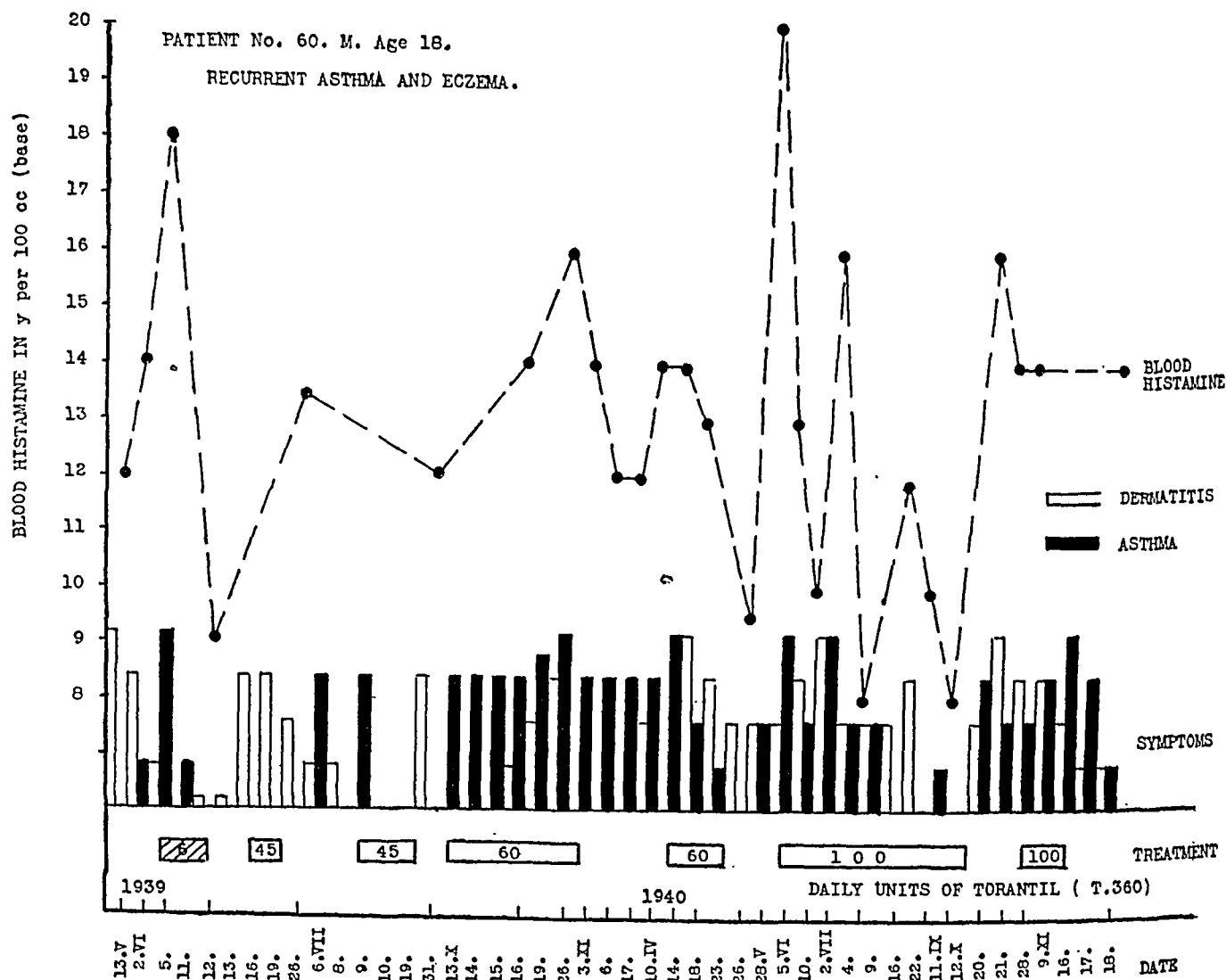


FIG. 3. THE HISTAMINE CONTENT OF THE BLOOD OF A PATIENT WITH CHRONIC ASTHMA AND ECZEMA TAKEN OVER A PERIOD OF ABOUT TWO YEARS

The determinations were made at random intervals both in and out of attack.

patients. However, there does not seem to be any consistent correlation of these changes with the onset or disappearance of symptoms. This is shown in Figure 2 where the symptoms and blood histamine levels of three patients with asthma are recorded both during severe attacks and during a symptom-free period.

One patient (Case 60) was followed at intervals in this manner for a period of two years. The results of the blood histamine determinations are shown along with symptoms in Figure 3. It will be noted that the histamine content of the blood fluctuated markedly without a consistent relationship to the symptoms of active asthma or dermatitis. Many of the attacks were associated with a high blood histamine level, such as that in June 1939 when the histamine content of the blood

rose to 18.0 y per 100 cc. as an acute attack of asthma came on, and fell to a low level on June 12th when symptoms disappeared. In October 1939, there was persistent asthma and severe eczema. The histamine content of the blood rose and fell without much obvious change in the symptoms. Again, a marked increase of the blood histamine level (20 y per 100 cc.) was observed in May 1940; there was only a slight increase in symptoms of asthma. On June 5th he returned with severe asthma and the histamine content of the blood was only 10.0 y per 100 cc. At the last visit made on February 14th, 1941, this patient was completely free of asthma, and there was a moderate amount of eczema. The blood histamine content was 22.0 y per 100 cc., higher than at any previous time. It would seem that the symptoms

TABLE II

*Blood histamine in cases of uncomplicated urticaria*

Case	Sex	Age	Blood histamine <i>γ per 100 cc.</i>	Symptoms
15	F	24	1.7	0
28	F	14	4.0	0
			4.0	++
48	F	24	3.0	0
			2.0	++
59	F	25	4.0	0
			4.0	++
			3.0	++++
61	F	18	2.0	++++
			3.5	0
63	M	24	8.0	++
			7.0	++++
97	F	17	7.0	0
			6.0	++
189	M	10	4.0	++
			5.0	0
			4.0	++++
			4.0	0
178	F	40	3.0	++
			4.2	0
51	F	40	8.0	0
121	F	30	6.0	0
			6.0	++
			6.0	0
			11.0	++++
133	F	48	3.5	+++
			3.8	++
151	F	22	6.0	0
174	F	23	6.0	0
			3.0	0
			4.0	++
202	F	36	3.0	+++
214	F	60	1.5	++
217	F	23	5.5	+++
259	M	3	6.8	0
			6.6	++

of allergy are not associated with any consistent change in the histamine content of the blood in this patient.

### (b) Cases of uncomplicated urticaria

Twenty-three patients with recurrent urticaria were available for study. For the most part, no difference in the histamine content of the blood was noted as compared to that of normal persons, as will be seen in Table II. In one case only (Case 121) a moderate increase was observed during an acute attack. In the remaining twenty-two patients, minor fluctuations of the blood histamine level occurred, and these were usually in the nature of a small decrease coincident with an attack of urticaria. These findings, therefore, are not in agreement with those of Cerqua (13) who noted an increase of the histamine content of the blood during attacks of urticaria.

TABLE III

*Blood histamine in cases of angioneurotic oedema*

Case	Sex	Age	Date	Blood histamine <i>γ per 100 cc.</i>	Symptoms
37	M	9		2.0	++
				0.5	++++
73	F	22		3.0	0
79	F	12		1.0	+++
87	M	21		0.5	After severe attack (24 hours symptoms receding) 0
89	F	26		5.0	+++
				0.5	++
91	M	22		1.0	++
				1.0	36 hours after (subsiding) Severe attack +++++
21	F	24	June 13, 1940	0.8	0
219	F	27	July 22, 1940	5.0	++++
			September 9, 1940	0.5	++
			October 11, 1940	1.5	+
			November 21, 1940	3.0	No oedema, but complaining of blurred vision
				0.7	

### (c) Cases of angioneurotic oedema

The patients susceptible to attacks of acute angioneurotic oedema, of whom there were eight, showed the most consistent alterations in the histamine content of the blood. The results are given in Table III. It will be noted that in each instance, with the exception of Case 37, a moderate-to-marked decrease in the blood histamine occurred when acute symptoms were present. This was observed in repeated attacks in two patients. For example, in Case 89, the blood histamine level was low when symptoms were marked, and returned to within normal levels as they decreased (Figure 4).

TABLE IV

*Blood histamine in cases of eczema*

Case	Sex	Age	Blood histamine <i>γ per 100 cc.</i>	Symptoms
43	F	38	4.0	++
72	F	34	4.0	+
90	F	6	9.0	++++
			8.0	+++
92	M	20	9.0	++
98	M	7	6.0	+++
101	F	40	10.0	+++
179	F	10	8.0	+
190	F	40	5.0	++
220	M	47	28.0	++++ (weekly intervals)
			12.0	++++
			13.0	++++
60	M	18	14.0	++

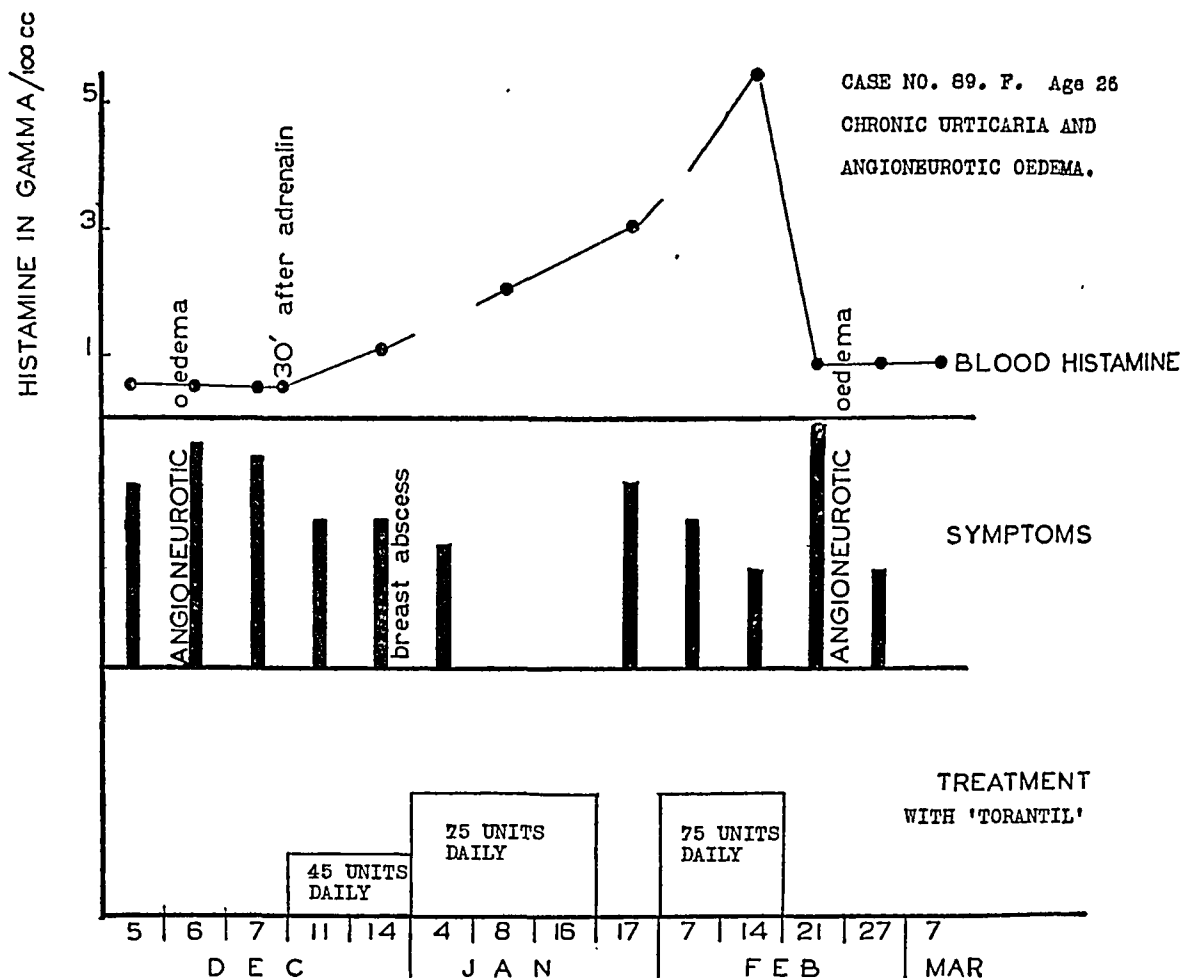


FIG. 4. THE HISTAMINE CONTENT OF THE BLOOD OF A PATIENT SUBJECT TO URTICARIAL ATTACKS AND ANGIONEUROTIC OEDEMA

It will be seen that coincident with an attack of angioneurotic oedema, a marked diminution of the histamine content of the blood occurs.

#### (d) Cases of eczema

The blood histamine level was determined in ten cases of eczema (Table IV). Unfortunately, single determinations only were made on most of these patients. It will be seen from the results that the blood histamine tends to be at levels higher than normal. Case 60, who was described previously with the group of asthmatic patients, is included. The histamine level of the blood was raised when eczema alone was present. In Figure 5, determinations of the blood histamine level in patients with eczema, urticaria and angioneurotic oedema are shown.

#### (c) Vasomotor rhinitis and colitis

There remains a small group of patients, six with vasomotor rhinitis, and two with colitis. The results of the blood histamine determinations

are given in Table V. Here again, it will be noted, there is little difference in the blood histamine level of patients with vasomotor rhinitis as compared to normals. In one of the cases of colitis (Case 153) the histamine content of the blood is definitely increased and there are marked fluctuations which are not associated with increase or decrease of symptoms.

The results of treatment, indicated on some of the figures, will be discussed elsewhere.

#### DISCUSSION

In reviewing the above results, several points may be noted. It will be seen that, in contrast to the marked stability of the blood histamine level of non-allergic or normal patients, that of patients with allergic disease, with the exception of urticaria, may vary considerably in the same person.

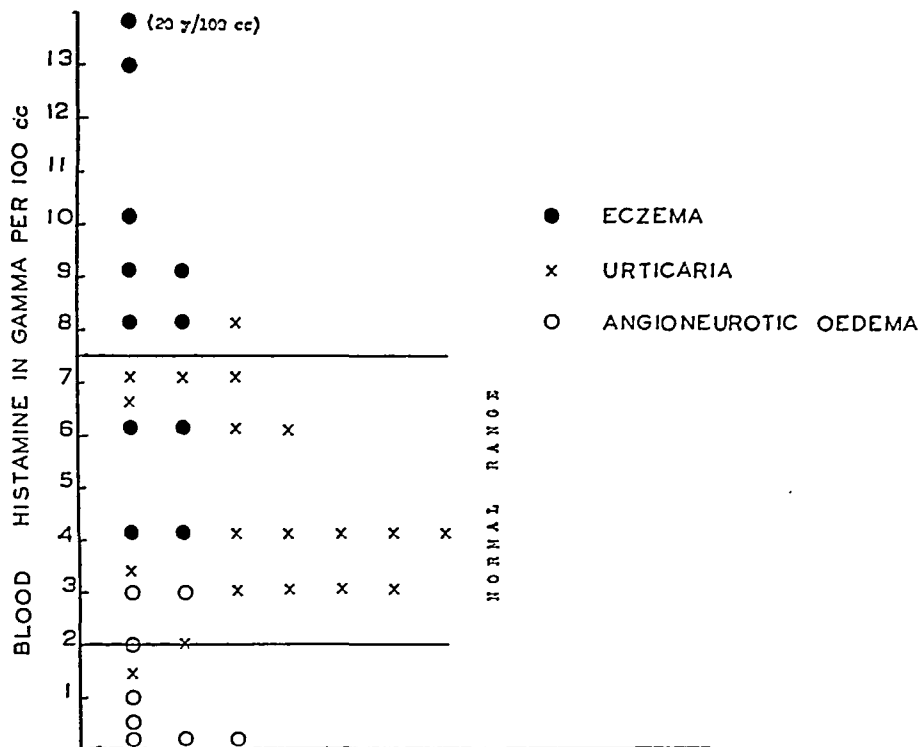


FIG. 5. THE HISTAMINE CONTENT OF THE BLOOD OF PATIENTS WITH ECZEMA, URTICARIA AND ANGIONEUROTIC OEDEMA

It will be observed that the histamine content of the blood of many of the patients with eczema tends to be at levels higher than normal, whereas that of patients with angioneurotic oedema is at a level below that of normal. Patients with urticaria, on the other hand, have a histamine content of the blood within normal limits.

Furthermore, the histamine content of the blood of allergic patients may increase considerably above the normal, as in young asthmatics or in cases of eczema, or may almost entirely disappear, as in cases of angioneurotic oedema. In this connection, it is interesting to note that Cerqua (13) also observed that the blood histamine level decreased in patients with serum sickness during attacks, and that it later returned to normal. Such a decrease has also been observed in cases of asthma where the attack was induced by the subcutaneous injection of histamine (22) and in cases of physical allergy following the production of wheals by the proper stimulus (20).

It is important, however, to distinguish between the fluctuations in the blood histamine level in patients with angioneurotic oedema, and those which occur in other types of allergic disease, for it is only in the former that the change in the

histamine content of the blood is definitely correlated with the onset of symptoms, while in the latter group of cases, such as eczema or asthma, fluctuations in the histamine content of the blood may or may not occur at the time of onset of symptoms. Furthermore, the blood histamine level always decreases in cases of angioneurotic oedema with the onset of symptoms, whereas in the other types of allergy, either an increase or a decrease may be observed.

The observations of Myhrman and Tomenius (23) are interesting in connection with the relation of histamine to asthma. They found that, whereas the stools of most patients contained relatively small amounts of histamine, averaging 1.3 γ per cc., those of asthmatic patients contained large amounts. In one patient, for example, the histamine content of the stool was found to be 256 γ per cc. and 100 γ per cc. was found in the



TABLE V

*Blood histamine in cases of vasomotor rhinitis and colitis*

Case	Sex	Age	Diagnosis	Date	Blood histamine <i>y</i> per 100 cc.	Symptoms
102	F	25	Vasomotor rhinitis		8.0	++
77	F	39	Vasomotor rhinitis		6.0	++
					7.0	++++
					12.0	++++
69	F	33	Vasomotor rhinitis		4.0	0
78	F	14	Vasomotor rhinitis	April 27, 1940	3.5	++
				May 13, 1940	2.5	++
				May 23, 1940	10.0	++++
				June 5, 1940	3.0	++
				June 8, 1940	5.0	++++
				June 13, 1940	3.0	+++
				July 11, 1940	3.0	++++
207	F	29	Vasomotor rhinitis		8.0	+++
225	M	25	Colitis		8.0	+++
153	M	20	Colitis	April 13, 1940	8.0	++
				May 23, 1940	14.0	+
				May 31, 1940	10.5	+
				June 15, 1940	14.0	++++
				June 20, 1940	9.0	++++
				June 21, 1940	6.5	++++
				June 28, 1940	8.0	++++
				July 8, 1940	7.0	++++

stool of another. On the other hand, no increase was noted in cases of urticaria. Histamine-like substances have also been found in the sputa of asthmatic patients (17, 24).

While it is as yet not possible to account for the fluctuations in the blood histamine level or to explain its relationship to allergic disease, a consideration of the state of the blood histamine and its behaviour following the injection of histamine may somewhat clarify these findings. It is agreed by the majority of observers that most of the blood histamine is held within the cellular elements—the white blood cells (19) or the platelets (25, 26). Thus an increase of such histamine-rich cells would account for an increase in the total blood histamine. Code and MacDonald (19) reported five patients with an eosinophilia of over 9 per cent, in four of whom the histamine content of the blood was raised above normal. The results of the present investigation support this finding in general, for the majority of patients in whom the blood histamine was found to be increased also had an eosinophilia of from 10 to 20

per cent. This parallelism, however, was not invariable. For example, one patient (Case 273), a case of Loeffler's syndrome, had an eosinophilia of 60 per cent on one occasion, and of 55 per cent on another. The blood histamine level on both, respectively, was 2.0 *y* per 100 cc. Similarly, Case 43, Table IV, had an eosinophilia of 15 per cent. The histamine content of the blood was 4.0 *y* per 100 cc.

It should, however, be noted that marked increases in the histamine content of the whole blood may exist without any symptoms being manifest. Thus, in cases of myelogenous leukemia, as much as 1500 *y* per 100 cc. have been reported. Such histamine is probably bound to the cells (19, 27). On the other hand, a sudden liberation of histamine into the blood may produce a rapid and severe fall of the blood pressure and tachycardia. Even as small an amount as 7.0 gamma, when rapidly injected intravenously, will produce such changes (28).

It is probable that, in certain cases of physical allergy, histamine may be released free into the plasma and there may produce a decrease in the blood pressure and tachycardia along with a release of free hydrochloric acid into the stomach (11). Actual increases in the blood histamine have been observed in one such case by Capps and Young (29) and in eight cases by Rose (20). Such increases are transient and are frequently followed by a decrease in the blood histamine level within twenty to thirty minutes. It is probable that this histamine is in the plasma rather than in the cells and, as such, would rapidly be withdrawn from the circulation (30). This is further supported by the observation that, following the subcutaneous injection of histamine in allergic patients, the histamine level of the blood may remain at a stationary level, or may decrease even at the height of the symptoms of histamine intoxication, such as tachycardia, headache or allergic manifestations (22). Thus, even when histamine is liberated, it is possible that the tissue affected may absorb the free histamine and withdraw it from the circulating blood. This may possibly account for the lack of correlation between the blood histamine levels and symptoms in other forms of allergic disease, as noted above. It would also ac-

count in part for the decrease in the blood histamine level in cases of angioneurotic oedema. If it is believed that histamine is carried by the eosinophiles, the finding that there is a gradual increase of these cells in wheals (31) would also account for the decrease in the blood histamine.

Finally, it is possible that human allergy may resemble both types of animal anaphylaxis, with a liberation of histamine in certain types and a decrease of the histamine content of the blood in others.

### CONCLUSIONS

1. The histamine content of the blood of a group of eighty patients with allergic disease including asthma, urticaria, angioneurotic oedema, eczema, vasomotor rhinitis and colitis has been studied.

2. With the exception of cases of urticaria, there is much more fluctuation of the histamine content of the blood of patients with allergic disease, as compared to the marked stability of that of normal persons.

3. In cases of asthma, eczema or vasomotor rhinitis, these fluctuations are not necessarily correlated with the onset of symptoms.

4. With the development of angioneurotic oedema, a marked diminution of the histamine content of the blood occurs. The blood histamine level returns to normal when the symptoms subside.

5. Although it is possible that histamine may be released in the types of allergic disease studied in the present communication, the results of the above observations do not warrant such a conclusion.

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TABLE V

*Blood histamine in cases of vasomotor rhinitis and colitis*

Case	Sex	Age	Diagnosis	Date	Blood histamine <i>y per 100 cc.</i>	Symptoms
102	F	25	Vasomotor rhinitis		8.0	++
77	F	39	Vasomotor rhinitis		6.0	++
					7.0	++++
					12.0	++++
69	F	33	Vasomotor rhinitis		4.0	0
78	F	14	Vasomotor rhinitis	April 27, 1940	3.5	++
				May 13, 1940	2.5	++
				May 23, 1940	10.0	++++
				June 5, 1940	3.0	++
				June 8, 1940	5.0	++++
				June 13, 1940	3.0	+++
				July 11, 1940	3.0	++++
207	F	29	Vasomotor rhinitis		8.0	+++
225	M	25	Colitis		8.0	+++
153	M	20	Colitis	April 13, 1940	8.0	++
				May 23, 1940	14.0	+
				May 31, 1940	10.5	+
				June 15, 1940	14.0	++++
				June 20, 1940	9.0	++++
				June 21, 1940	6.5	++++
				June 28, 1940	8.0	++++
				July 8, 1940	7.0	++++

stool of another. On the other hand, no increase was noted in cases of urticaria. Histamine-like substances have also been found in the sputa of asthmatic patients (17, 24).

While it is as yet not possible to account for the fluctuations in the blood histamine level or to explain its relationship to allergic disease, a consideration of the state of the blood histamine and its behaviour following the injection of histamine may somewhat clarify these findings. It is agreed by the majority of observers that most of the blood histamine is held within the cellular elements—the white blood cells (19) or the platelets (25, 26). Thus an increase of such histamine-rich cells would account for an increase in the total blood histamine. Code and MacDonald (19) reported five patients with an eosinophilia of over 9 per cent, in four of whom the histamine content of the blood was raised above normal. The results of the present investigation support this finding in general, for the majority of patients in whom the blood histamine was found to be increased also had an eosinophilia of from 10 to 20

per cent. This parallelism, however, was not invariable. For example, one patient (Case 273), a case of Loeffler's syndrome, had an eosinophilia of 60 per cent on one occasion, and of 55 per cent on another. The blood histamine level on both, respectively, was 2.0 *y per 100 cc.* Similarly, Case 43, Table IV, had an eosinophilia of 15 per cent. The histamine content of the blood was 4.0 *y per 100 cc.*

It should, however, be noted that marked increases in the histamine content of the whole blood may exist without any symptoms being manifest. Thus, in cases of myelogenous leukemia, as much as 1500 *y per 100 cc.* have been reported. Such histamine is probably bound to the cells (19, 27). On the other hand, a sudden liberation of histamine into the blood may produce a rapid and severe fall of the blood pressure and tachycardia. Even as small an amount as 7.0 gamma, when rapidly injected intravenously, will produce such changes (28).

It is probable that, in certain cases of physical allergy, histamine may be released free into the plasma and there may produce a decrease in the blood pressure and tachycardia along with a release of free hydrochloric acid into the stomach (11). Actual increases in the blood histamine have been observed in one such case by Capps and Young (29) and in eight cases by Rose (20). Such increases are transient and are frequently followed by a decrease in the blood histamine level within twenty to thirty minutes. It is probable that this histamine is in the plasma rather than in the cells and, as such, would rapidly be withdrawn from the circulation (30). This is further supported by the observation that, following the subcutaneous injection of histamine in allergic patients, the histamine level of the blood may remain at a stationary level, or may decrease even at the height of the symptoms of histamine intoxication, such as tachycardia, headache or allergic manifestations (22). Thus, even when histamine is liberated, it is possible that the tissue affected may absorb the free histamine and withdraw it from the circulating blood. This may possibly account for the lack of correlation between the blood histamine levels and symptoms in other forms of allergic disease, as noted above. It would also ac-

count in part for the decrease in the blood histamine level in cases of angioneurotic oedema. If it is believed that histamine is carried by the eosinophiles, the finding that there is a gradual increase of these cells in wheals (31) would also account for the decrease in the blood histamine.

Finally, it is possible that human allergy may resemble both types of animal anaphylaxis, with a liberation of histamine in certain types and a decrease of the histamine content of the blood in others.

### CONCLUSIONS

1. The histamine content of the blood of a group of eighty patients with allergic disease including asthma, urticaria, angioneurotic oedema, eczema, vasomotor rhinitis and colitis has been studied.

2. With the exception of cases of urticaria, there is much more fluctuation of the histamine content of the blood of patients with allergic disease, as compared to the marked stability of that of normal persons.

3. In cases of asthma, eczema or vasomotor rhinitis, these fluctuations are not necessarily correlated with the onset of symptoms.

4. With the development of angioneurotic oedema, a marked diminution of the histamine content of the blood occurs. The blood histamine level returns to normal when the symptoms subside.

5. Although it is possible that histamine may be released in the types of allergic disease studied in the present communication, the results of the above observations do not warrant such a conclusion.

The author wishes to thank Dr. J. S. L. Browne for his valuable criticism and suggestions. Thanks are also due to Dr. A. T. Henderson and the Department of Allergy of the Royal Victoria Hospital and to Dr. H. Bacal and the Department of Allergy of the Children's Memorial Hospital for providing many of the patients, and to Mrs. E. V. Harkness for technical assistance.

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# MAGNESIUM PARTITION STUDIES IN GRAVES' DISEASE AND IN CLINICAL AND EXPERIMENTAL HYPOTHYROIDISM

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*(Received for publication April 1, 1941)*

In a previous paper (1) we reported the results of determinations of total magnesium and ultrafiltrable magnesium in the serum of patients with hyperthyroidism. These findings were contrasted with those obtained in normal individuals, patients with autonomic imbalance, and patients with various types of muscular dystrophy. It was demonstrated that in normal individuals the percentage of the total serum magnesium which is non-diffusible did not exceed 22.1 per cent, with an average of 14.5 per cent, although Watchorn and McCance (2) report that, normally, approximately 25 per cent of the total serum magnesium is bound. The results obtained in patients with autonomic imbalance and various muscular dystrophies fell quite well within the normal range. In contrast to these groups, the patients with hyperthyroidism showed a very marked increase in the percentage of bound magnesium which varied from 21.5 to 61.6 per cent, with an average of 36.0 per cent. It was further pointed out that, after adequate treatment with iodine, there occurred a drop in the percentage of the non-diffusible magnesium fraction, which finally reached normal levels after subtotal thyroidectomy. No correlation was found between the individual basal metabolic rates and the percentage of bound magnesium. This is perhaps due to the fact that the metabolic rate was determined very shortly after admission to the hospital ward and hence may not represent the true basal rate. The increase in the percentage of non-diffusible magnesium in Graves' disease occurred entirely at the expense of the diffusible fraction, since the total serum magnesium in the patients with hyperthyroidism was approximately the same as that of the normal controls.

In the present paper we are reporting further results of studies of magnesium partition in pa-

tients with hyperthyroidism, as well as in individuals with myxedema. We are also including experimental data on totally thyroidectomized dogs.

## METHOD

The total serum magnesium was determined by the method of Briggs (3). The serum proteins were precipitated with trichloroacetic acid. It was found that little, if any, magnesium was carried down with the protein flocculum. To 10 cc. of protein-free filtrate were added 1 cc. of 20 per cent sodium acetate, 6 to 8 drops of 0.016 per cent bromocresol green, and 1 cc. of 4 per cent ammonium oxalate. The pH of the solution was adjusted to 5.0 by addition of ammonium hydroxide. The mixture was allowed to stand overnight, and the precipitated calcium oxalate was then separated by centrifugation. To the decanted supernatant fluid and washings were added 1 cc. of a 2 per cent potassium dihydrogen phosphate, and 1 cc. of concentrated ammonia solution. After the mixture had again been allowed to stand overnight, the precipitate was separated by centrifugation and washed with a solution containing 200 cc. of 95 per cent alcohol and 50 cc. of concentrated ammonia solution per liter. The precipitated magnesium ammonium phosphate was dried and determined according to the method of Kuttner and Lichtenstein (4) by comparison of the color developed on addition of 7.5 per cent sodium molybdate and 0.2 per cent stannous chloride with that of a standard phosphate stock solution.

For the determination of diffusible magnesium, serum was ultrafiltered through a "600" cellophane membrane under a pressure of 80 pounds of nitrogen per square inch. The magnesium content of the ultrafiltrate was determined as described above, except that the protein precipitate with trichloroacetic acid was omitted.

## RESULTS

In Table I are presented the data obtained on 50 patients with well-defined hyperthyroidism. In each instance, the diagnosis was confirmed by microscopic study of the excised gland. Of this group of 50 patients, the percentage of bound serum magnesium fell within the normal range level in 6, while it varied between 20 and 25 per

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TABLE I

*Distribution of magnesium in cases of hyperthyroidism and relation to average basal metabolic rate*

Percentage of bound magnesium (Serum)	Number of patients	Average basal metabolic rate
<i>per cent</i>		
20 or less	6	+36
20 to 25	9	+43
25 to 30	7	+49
30 to 40	18	+44
40 and over	10	+45

cent in an additional 9 instances. These latter results were classified as borderline. In the remaining 35 patients the percentage of non-diffusible magnesium was considerably above the normal level.

We attempted to correlate the duration and severity of the illness with the percentage of bound magnesium. For this purpose the data (Table II) were classified according to three time intervals: (a) 0 to 3 months, (b) 3 to 24 months, and (c) more than 24 months. In general, we find no statistically significant difference in magnesium distribution between classes (a) and (b). However, a comparison of (b) and (c) reveals

TABLE II

*Relationship of percentage of bound magnesium\* to duration of symptoms and to severity of disease*

Percentage of bound magnesium (Serum)	Total number of patients	Number of Patients				
		Duration of symptoms months			Severity of symptoms	
		0-3 (a)	3-24 (b)	24 or more (c)	Mild	Moderate and severe
Less than 25 per cent	15	4	5	6	11	4
More than 25 per cent	35	7	24	4	4	31

\* In order to establish the validity of the correlation inferred from Table II, the data were subjected to contingency analysis. From the data for "low" (less than 3 months) and "average" (3 to 24 months) duration of Graves' disease associated with "normal" (0 to 25 per cent) and "abnormal" (greater than 25 per cent) values for per cent of bound magnesium, Pearson's coefficient of mean square contingency ( $C$ ) = 0.2 and  $\chi^2$  = 1.67, which yields a value of  $P$  slightly less than 0.20. This value of  $P$  is so large as to afford no evidence of correlation. From the analogous data for "average" and "high" (more than 24 months) duration,  $C$  = 0.38,  $\chi^2$  = 6.71, and  $P$  = 0.01. In this case, the evidence for correlation is significantly better, the odds being more than 99 to 1 in its favor.

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that the percentage of bound serum magnesium tends to be significantly lower in patients with symptoms of more than 2 years' duration than in the other group (see footnote to Table II). Similarly, an increase in the non-diffusible magnesium is associated with an increase in the severity of the disease—a relation which is directly apparent from the data.

After treatment with iodine there occurs a distinct drop in the percentage of bound serum magnesium, which is lowered further after operation (Table III). In several instances, the non-ultrafiltrable magnesium level in the blood fell to 0.0 per cent. In these instances, the postoperative basal metabolic rate fell to myxedematous levels.

TABLE III

*Effect of treatment with iodine and operation on the percentage of bound magnesium*

Number of cases	Average percentage of bound magnesium (serum)		
	Control	After iodine treatment	After operation
25*	30.6	18.9	14.3
21†	30.9		14.5
15	29.5	18.1	

\* In 3 patients there was no appreciable drop in the percentage of bound magnesium after iodine treatment.

† In 3 patients there was no appreciable drop in the percentage of bound magnesium after operation.

In Table IV are presented the data obtained on patients with myxedema. In the 7 instances reported, the percentage of bound serum magnesium was markedly below the normal level, a finding which is the direct antithesis of that observed in Graves' disease. In 3 of the 7 patients, studies were subsequently made after adequate treatment with thyroid extract. In 2 of the 3 instances there occurred an increase in the percentage of bound serum magnesium which approximated the normal values.

We attempted to duplicate these results experimentally. For this purpose total thyroidectomies were performed on 4 dogs. Serum magnesium partition studies were conducted before, and repeatedly after the operation. In each instance, within 17 days to 5 weeks after operation there occurred a profound drop in the percentage of bound magnesium. In dog 181 the bound magnesium could be raised to normal levels by the administration of thyroxine. When the therapy was

TABLE IV

*Serum magnesium studies in patients with myxedema*

Name	Basal metabolic rate	Total magnesium	Ultra-filtrable magnesium	Percentage of magnesium bound
		mgm. per cent	mgm. per cent	
BEFORE TREATMENT WITH THYROID EXTRACT				
E. A.	-30	2.64	2.73*	0
N.	-30	1.62	1.60	0
A. C.	-33	2.16	2.17	0
R. B.	-32	2.43	2.29	5.7
F.	-30	2.32	2.79*	0
G. G.	-33	2.43	2.36	2.8
G. B.	-22	2.60	2.61	0
AFTER TREATMENT WITH THYROID EXTRACT				
E. A.	+2	2.31	2.02	12.5
N.	+7	1.66	1.49	10.2
R. B.	+7	2.35	2.19	5.2

\* These values fall below the zero level because of certain technical artefacts which, when corrected, bring the results to the zero level.

discontinued, the non-diffusible magnesium returned to the previous low postoperative level. These changes in bound magnesium could be induced at will by the administration and withdrawal of thyroxin in totally thyroidectomized animals (Table V).

## DISCUSSION

In a large percentage of patients with Graves' disease there occurs a marked increase in the percentage of bound serum magnesium. In patients with well-defined hyperthyroidism in whom the non-diffusible magnesium was normal, certain factors operated which contributed to the normal levels obtained. Many patients with Graves' disease were treated extensively prior to admission to the hospital. Thus, of our group of 15 patients whose bound magnesium was normal or borderline, most had received some medication prior to admission to the hospital ward. It is, of course, difficult to determine the nature of such medication, but it is not inconceivable that iodine, iodides, or iodized salt was part of the therapy employed. Since the use of iodine tends to lower the percentage of bound magnesium, this may have played a part in those instances in which normal values were obtained. It is interesting that patients with unduly prolonged Graves' disease tend

to yield normal magnesium partition studies. The reasons for this are at present obscure, but it appears that these changes are mediated through the thyroid and occur only after certain alterations in thyroid function have occurred. This is further supported by the fact that, after thyroidectomy in patients with Graves' disease, the magnesium partition tends to return to a normal pattern. Similarly, in patients with myxedema, or

TABLE V

*Data on totally thyroidectomized dogs*

Date	Total serum magnesium	Percentage magnesium bound	Remarks
	<i>mgm. per cent</i>		
Dog (181)			
March 22, 1940	2.04	4.9	April 1, thyroidectomy
April 8.....	2.14	3.7	
April 23.....	2.16	2.7	
			May 1, thyroxin 2 mgm. daily intramuscularly begun
May 10.....	2.37	15.6	May 21, thyroxin stopped
May 21.....	2.15	23.2	
June 4.....	2.08	25.4	
June 17.....	2.11	21.8	
July 2.....	2.11	5.2	
July 13.....	2.07	0.0	
September 10..	3.51	26.8	Dog seriously ill, almost moribund
			September 10, thyroxin 2 mgm. daily begun
September 23..	2.25	0.0	
October 11..	2.37	0.0	
October 21..	2.34	28.2	
Dog (277)			
May 13.....	2.71	25.4	May 14, thyroidectomy
May 21.....	2.28	20.5	
June 17.....	2.32	27.5	
July 2.....	2.22	5.8	Died
July 13.....	2.56	41.0*	
Dog (288)			
June 3.....	2.32	21.5	June 6, thyroidectomy
June 20.....	2.34	34.1	
June 27.....	1.78	35.4	
July 11.....	1.93	0.0	
July 13.....	2.02	0.0	
Dog (292)			
June 21.....	2.07	23.3	June 20, thyroidectomy
July 3.....	1.94	5.6	
July 8.....	2.20	0.0	
July 13.....	3.26	34.3*	Died

\* These determinations were performed on blood drawn a few minutes before the death of the animals, when there apparently occurs a spontaneous preagonal increase in the percentage of bound magnesium.

in the totally thyroidectomized dog, the non-diffusible serum magnesium can be altered by the administration or withdrawal of thyroid extract. Here, too, a considerable period of time must elapse after administration or cessation of therapy before shift in the ionizable—non-ionizable magnesium ratio occurs. Such magnesium changes do not occur when thyroid extract is administered to dogs with intact thyroids or to normal individuals.

#### SUMMARY

1. Serum magnesium partition studies were conducted on 50 patients with proven Graves' disease.

2. In 6 of these patients the percentage of bound serum magnesium fell within the normal range level, that is 20 per cent or less. In 9 additional patients the results were borderline, that is the percentage of bound magnesium varied between 20 and 25 per cent. In 35, there was a definite increase in the non-diffusible magnesium. The bound fraction in these patients varied between 25 and 61.6 per cent of the total serum magnesium.

3. There is no definite correlation between the percentage of bound serum magnesium and the basal metabolic rate.

4. There is a definite relationship between the duration of the illness and the magnesium partition. Thus, in patients with symptoms of Graves' disease of more than 2 years, the non-diffusible fraction frequently tends to approximate normal levels.

5. After adequate treatment with iodine there occurs a marked drop in the percentage of bound serum magnesium, which is further lowered after subtotal thyroidectomy.

6. In patients with myxedema, as well as in totally thyroidectomized dogs, the non-diffusible serum magnesium fraction is extremely low and frequently is 0 per cent. This is the direct antithesis of the results obtained in Graves' disease.

7. After administration of thyroid extract or thyroxin to the myxedematous patients, and to the thyroidectomized dogs, the percentage of bound serum magnesium returns to approximately normal levels.

8. The increase or decrease of the percentage of bound magnesium occurs at the expense of the ionizable fraction, since the total serum magnesium remains unaltered.

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PROCEEDINGS OF THE THIRTY-THIRD ANNUAL MEETING OF  
THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION  
HELD IN ATLANTIC CITY, N. J., MAY 5, 1941

READ BEFORE THE SCIENTIFIC SESSION

*The Relation of Sulfanilamide "Acidosis" to the Specific Inhibition of Carbonic Anhydrase.* By W. BARRY WOOD, JR. and CUTTING B. FAVOUR (introduced by P. H. Long), Baltimore, Md.

The cause of the "acidosis" which accompanies sulfanilamide therapy is not known. Mann and Keilin suggested a possible relationship between the acid-base disturbance and inhibition of carbonic anhydrase. This enzyme, contained in red blood cells, catalyzes the reaction  $\text{H}_2\text{CO}_3 \rightleftharpoons \text{CO}_2 + \text{H}_2\text{O}$ , and was shown to be specifically poisoned *in vitro* by unsubstituted sulfonamide derivatives ( $\text{R-SO}_2\text{NH}_2$ ).

Using the manometric method of Meldrum and Roughton, we have extended the studies of Mann and Keilin and have made the following observations which suggest that sulfanilamide "acidosis" is due to inhibition of carbonic anhydrase:

1. Sulfanilamide added to blood *in vitro* (10 mgm. per cent) reduced the enzymatic activity of the red cells to that of normal blood diluted 100 times. Sulfapyridine, sulfathiazole, and sulfadiazine did not affect the enzyme.

2. The bloods of all patients receiving sulfanilamide showed low  $\text{CO}_2$  combining powers and marked depression of carbonic anhydrase; the bloods of patients taking sulfapyridine, sulfathiazole, and sulfadiazine were normal.

3. By dialysis experiments *in vitro* the reaction between sulfanilamide and carbonic anhydrase was shown to be reversible.

4. When sulfanilamide was injected intravenously into dogs, inhibition of carbonic anhydrase and a marked fall in arterial  $\text{CO}_2$  content occurred within two minutes. The lowering of the  $\text{CO}_2$  content consistently followed inactivation of the enzyme.

*Human Sulfathiazole Sensitivity. Observations upon the Febrile, Leukocytic and Immunologic Response.* By THEODORE J. ABERNETHY, SAMUEL C. BUKANTZ, and JOHN MINOR (introduced by Theodore G. Klumpp), Washington, D. C.

Single doses of sulfonamide drugs were administered to a patient exhibiting fever, rash, and leukocytosis during treatment of lobar pneumonia with sulfathiazole. Two grams of sulfathiazole, on the 6th day of normal temperature following cessation of therapy, induced fever and marked leukocytosis. Appreciable amounts of the drug were detected in the blood 2 hours after its oral administration, but the febrile and leukocytic reactions were delayed for 4 hours. Other blood studies were normal. Similar doses of sulfanilamide and sulfamethylthiazole, although accompanied by identical blood concentration, induced no reaction, while response to sulfapyridine was minimal. That sensitivity had been retained during these negative responses was indicated by the persistence of re-

sponse to as small a dose of sulfathiazole as 0.5 gram, given subsequently.

Skin tests of the sensitive patient, using saline solutions of the pure drugs, were negative. Attempts have been made to detect antibodies to coupled products of diazotized sulfathiazole and serum albumin, globulin, or resorcinol. Precipitation of these azo-antigens with the sensitized patient's serum, as well as certain unrelated immune sera, has been found to occur. Observations upon the specificity of this reaction and its application to the investigation of toxic manifestations to sulfonamides have been made.

*Experimental and Clinical Studies on Gramicidin.* By WALLACE E. HERRELL and DOROTHY HEILMAN (introduced by Dr. B. T. Horton), Rochester, Minn.

A bactericidal substance isolated by Dubos (J. Exper. Med., 1939, 70, 1; Ann. Int. Med., 1940, 13, 2025) from a soil bacillus has a marked bactericidal action against gram-positive bacteria. This substance is toxic for laboratory animals when administered by the intravenous route. We have recently shown that one of the toxic effects of this substance is its hemolytic activity. The crude substance (tyrothricin) consists of two fractions, tyrocidine and gramicidin, as reported by Hotchkiss and Dubos (J. Biol. Chem., 1940, 136, 803). They found gramicidin to be the more active against the gram-positive bacteria.

Further studies in our laboratory have shown that the hemolytic effect of the crude substance is due to the presence of gramicidin. Using the tissue culture technic, we have determined the amounts of tyrocidine and gramicidin necessary to inhibit the growth of a number of strains of common gram-positive pathogenic bacteria. Small amounts of gramicidin (0.0005 to 0.0025 mgm.) inhibit the growth of all strains of pneumococci tested. Slightly larger amounts (0.005 to 0.01 mgm.) are required to inhibit strains of hemolytic streptococci, whereas still larger amounts are necessary to prevent growth of *Streptococcus faecalis*, *Streptococcus viridans*, and *Staphylococcus*. Tyrocidine is much less effective than gramicidin against all of these organisms. These results are drawn from approximately 2000 tissue culture preparations used in this study.

Clinical experiences with the local application of gramicidin in the treatment of infections caused by gram-positive bacteria are reported at this time. Suitable methods of applying this substance locally are also reported.

*Observations on the Use of "Gramicidin" (Dubos) in the Treatment of Streptococcal and Staphylococcal Infections.* By CHARLES H. RAMMELKAMP (by invitation) and CHESTER S. KEEFER, Boston, Mass.

Gramicidin is a bactericidal substance which was extracted from certain soil bacilli by René J. Dubos in 1939.

It was capable of killing large numbers of gram-positive bacteria *in vitro* and it was found to be capable of protecting mice which had been injected with fatal doses of pneumococci.

The present report deals with the use of this substance in the treatment of hemolytic streptococcal and Staphylococcus aureus infections. The following facts emerge: (1) Relatively large amounts of gramicidin could be injected into the serous cavities of rabbits without producing any toxic effects. (2) Hemolytic streptococcal empyema in rabbits could be cured following the injection of the material into the pleural cavity. Dosage and the time elapsing between the onset of infection and treatment were important in the outcome. (3) Staphylococcus aureus infections in closed cavities were much more resistant to treatment and larger doses were necessary. (4) As far as the evidence goes, it seems clear that superficial infections in man due to staphylococci and streptococci respond in a satisfactory manner following the local application of the material. The dosage and the frequency of application require further study.

*Penicillin as a Chemotherapeutic Agent.* By MARTIN H. DAWSON and (by invitation) GLADYS L. HOBBS, KARL MEYER and ELEANOR CHAFFEE, New York, N. Y.

Fleming (1929) observed that a certain strain of penicillium produced a substance which exerted a marked antibacterial action. The substance was named penicillin. Chain *et al* (1940) reported that they had been able to obtain a considerable yield of penicillin. They showed that the substance exerted a remarkable antibacterial effect both *in vitro* and *in vivo* against gram-positive organisms, including anaerobes of the gas gangrene group.

Preparations of penicillin have been made in our laboratory which, in dilutions of one gamma per cc. inhibit the growth of 2,500,000 hemolytic streptococci. The effect appears to be bactericidal. Penicillin is also effective against the following organisms: pneumococcus, Streptococcus viridans, Staphylococcus, *Cl. welchii*, *Cl. sporogenes* and *Cl. septicus*. Penicillin is likewise effective *in vivo*. Mice can be protected against 100,000,000 lethal doses of hemolytic streptococci. In the dosage employed little or no toxic effect has been observed. The methods of preparation are described, together with such information as is known concerning its chemical nature. Brief mention is made of the use of penicillin in human infections.

*A Study of Sulfonamide Inhibitors by the Use of a Soil Bacillus Which Decomposes p-Aminobenzoic Acid.* By GEORGE S. MIRICK (introduced by Colin M. MacLeod), New York, N. Y.

Substances inhibitory to the bacteriostatic action of the sulfonamide drugs are widely distributed in nature. *p*-aminobenzoic acid is an active inhibitor in quantities too small to be detected by the sensitive but non-specific diazo reaction. The great inhibitory activity of *p*-aminobenzoic acid raises the question of its identity with the other sulfonamide inhibitors.

A soil bacillus has been isolated which attacks *p*-aminobenzoic acid, destroying at the same time its diazo reaction and its activity as a sulfonamide inhibitor. The bacillus is quite specific, attacking both free and acetylated *p*-aminobenzoic acid, and *o*-aminobenzoic acid, but not *m*-aminobenzoic acid. It attacks novocaine but not the methyl and ethyl esters of *p*-aminobenzoic acid and none of the other related compounds tested except *p*-aminophenylacetic acid.

The bacillus has been used to study naturally occurring sulfonamide inhibitors. It destroys the inhibitory activity of yeast extract, which confirms other evidence that this activity is due to *p*-aminobenzoic acid. It does not inactivate the inhibitor present in peptone broth or a streptococcus extract. In fact, the bacillus itself forms or releases an inhibitor when grown in inhibitor-free liver infusion. The inhibitors present in broth and streptococci, and released by the bacillus in liver infusion, cannot be *p*-aminobenzoic acid since the microorganism destroys this compound.

*The Relation of Ascorbic Acid to Human Complement.*

By WESLEY W. SPINK and (by invitation) OLAF MICHELSEN and SUZANNE AGNEW, Minneapolis, Minn.

It is stated that the titer of complement in guinea pig serum is quantitatively related to the amount of reduced ascorbic acid present. It is claimed that a deficiency of serum complement may be corrected by the *in vivo* or *in vitro* addition of reduced ascorbic acid. The application of this knowledge to human beings is still a matter of controversy. In view of the importance of complement in the immune mechanism, and the emphasis placed upon the ascorbic acid requirements in human infections, we have investigated the relationship between complement and ascorbic acid in man.

Twenty-three patients having little or no reduced ascorbic acid in their plasmas were given large doses of ascorbic acid intravenously. Ascorbic acid and complement titrations carried out simultaneously on blood specimens obtained before and immediately after the injections revealed no change in complement titer. The hemolytic action of human complement in serums deficient in ascorbic acid could not be increased by the *in vitro* addition of the vitamin. Complement and ascorbic acid were found to be unrelated by other means. The activity of complement was shown to be independent of the presence of reduced ascorbic acid by oxidizing the acid without changing the titer of complement. Complementary activity was abolished by chemical and physical methods without interfering with the reduced ascorbic acid.

Thus far, our investigations have failed to establish a relationship between the hemolytic action of complement and ascorbic acid.

*The Effect of the Subcutaneous Injection of Histamine on the Histamine Content of the Blood of Patients With and Without Allergy.* By BRAM ROSE (by invitation) and J. S. L. BROWNE, Montreal, Canada.

In previous studies it was noted that the symptoms of allergy may be associated with marked fluctuations of the

histamine content of the blood, as compared to the marked stability of the blood histamine content of normal persons.

In order to investigate further the metabolism of histamine, the following procedure was carried out in 15 patients with allergic disease and in 15 non-allergic patients. The blood pressure, pulse and histamine content of the blood were first determined and again at five, fifteen and thirty minutes after the subcutaneous injection of 1 mgm. of histamine phosphate. Any other symptoms such as headache, flushing of the face, asthma or urticaria were also noted.

It was found that in cases of allergy, there was either no change, or a definite decrease in the blood histamine level, even when symptoms such as tachycardia, flushing of the face or headache were at their height. On the other hand, most of the non-allergic cases showed a definite increase in the blood histamine level when symptoms were pronounced, although a flat curve was also noted in several.

From these results it appears that the symptoms of histamine intoxication may be manifest in patients even though the histamine content of the blood remains at a stationary level or even decreases considerably.

The significance of these findings is discussed in relation to the changes in the histamine content of the blood in allergic disease and surgical shock.

*Spectrophotometric Study of Acute Hemolytic Anemia During Sulfonamide Therapy with Detection of a New Blood Pigment—Methemalbumin.* By CHARLES L. FOX, JR. (by invitation) and REUBEN OTTENBERG, New York, N. Y.

The acute hemolytic anemia of sulfanilamide and sulapyridine therapy was studied in four cases (two fatal) to determine the possible rôle of the abnormal blood pigments known to occur in this type of therapy.

Serum and urine containing the products of hemolysis and those erythrocytes which survived hemolysis were examined. Combined chemical methods and the objective Hardy recording spectrophotometer were utilized.

The red cells that survived hemolysis were essentially normal, containing but little methemoglobin. The urine contained very large amounts of hemoglobin and even larger amounts of methemoglobin up to 83 per cent. In the serum, representing the contents of the cells that underwent hemolysis, were found from 400 to 1700 mgm. per cent of three blood pigments: hemoglobin, methemoglobin and, contrary to expectation, not sulfhemoglobin but an entirely new pigment, methemalbumin.

This new pigment, recently discovered by Fairley, was prepared by incubating human hemoglobin with human serum. Its visible absorption spectrum (calculated on iron content) was obtained. This resembled methemoglobin except that in the red region a plateau-like maximum extended from  $\lambda 610\text{ m}\mu$  to  $\lambda 620\text{ m}\mu$ .

Analysis of the results permits these conclusions:

1. Sulfhemoglobin played no rôle.

2. The cells that survived hemolysis were almost entirely free of methemoglobin, whereas the cells that underwent hemolysis apparently contained proportions of methemoglobin somewhat greater than occur in the cells of other patients who do not suffer acute hemolytic anemia.

3. After hemolysis there is a rapidly progressive conversion of the hemoglobin and methemoglobin in the serum to methemalbumin. This conversion to a pigment which is not excreted in the urine may account for the fact that only a small portion (not over one-tenth) of the liberated hemoglobin and methemoglobin appeared in the urine and may explain the "conservation" of blood pigments. Of the 500 to 700 grams of pigments liberated by the acute hemolysis, nine-tenths were rapidly removed from the circulation by extra renal mechanisms.

*The Use of Purified Bovine Albumin Solutions as Plasma Substitutes.\** By CHARLES A. JANEWAY and PAUL B. BEESON. (introduced by Soma Weiss), with the technical assistance of Mary A. Bradley, Anne Shwachman, and Thomas Thurston, Boston, Mass.

The method of fractionation of plasma by equilibration with ethanol-water mixtures recently reported by Cohn and his associates makes possible the large scale preparation of purified bovine plasma protein fractions. In collaboration with Professor Cohn and his group, we are carrying out clinical and immunological investigations on these preparations, particularly the albumin, because its properties of low viscosity and great stability, solubility, and osmotic activity render it a theoretically ideal substance for increasing and maintaining plasma volume in acute emergencies.

Bovine albumin and gamma globulin, each prepared as one fraction in this method, approach homogeneity as measured by electrophoresis and in the ultracentrifuge, and show very slight immunological relationship to one another. Cross-reactions between anti-beef albumin sera and the ordinary therapeutic horse sera are slight and diminish with increasing purity of the albumin fraction.

Beef albumin solutions will maintain blood volume in dogs with shock induced by burns, and very little of the injected albumin escapes in the urine. In man, the intravenous injection of such solutions in varying amounts has been carried out in 16 subjects to date without untoward reactions, except for serum disease in one subject who had previously had serum sickness from horse serum. Much more work must be done before beef albumin can be evaluated as a therapeutic agent in shock, but the investigations in progress indicate that it can be administered safely to man and that it is retained in the blood stream satisfactorily.

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*Changes in Osmotic Pressure of Erythrocytes During Storage. Rôle in Causation of Transfusion Reactions.* By ELMER L. DEGOWIN and (by invitation) JOHN E. HARRIS, JOY BELL, and ROBERT C. HARDIN, Iowa City, Ia.

The amount of hemolysis in blood kept at 2° C. in the modified Rous-Turner dextrose-citrate preservative for 30 days was approximately one-half that occurring in citrated blood during 10 days of storage. Maximum inhibition of hemolysis during refrigeration required a concentration of at least 3 per cent dextrose in the blood mixture. The cell volume and the erythrocyte fragility gradually increased during the storage of citrated blood. When the blood-dextrose-citrate mixture was collected, the cell volume increased about 50 per cent with a corresponding change in fragility. This was due to diffusion of dextrose and water into the cells. The sugar could be washed out by physiologic saline, and normal fragility values could be obtained. There was an almost equivalent exchange between plasma sodium and cell potassium during storage but neither this nor the diffusion of chlorides, inorganic phosphorus, and lactic acid, nor pH changes could account for the secondary swelling.

When a 10 per cent dextrose solution was substituted for the 5.4 per cent concentration in the blood-dextrose-citrate mixture, the initial swelling of the erythrocytes was obviated. During storage the cell contents became so hypertonic that, when transfused, they were hemolyzed by the serum of the recipient. Sixteen hemolytic transfusion reactions were thus incurred. The excessive increase in osmotically active cell contents during preservation constitutes another criterion governing the limitation of storage. Over 2500 transfusions of blood stored in the 5.4 per cent dextrose-citrate solution have been administered. The storage limit of 30 days has proved satisfactory.

*Recent Advances in Clinical Medicine with the Aid of Artificially Prepared Radioactive Isotopes.* By JOHN H. LAWRENCE and (by invitation) JOSEPH G. HAMILTON, L. A. ERF, and CHARLES PECHER, Berkeley, Calif.

Radio-phosphorus has been shown to be selectively accumulated in the tissue infiltrated with leukemic cells of leukemic mice. The selective deposition of this radioactive substance in these tissues suggested that radio-phosphorus might be of therapeutic value in human leukemia since it appeared likely that the regions which had become infiltrated by leukemic cells would contain a relatively higher proportion of the administered radio-phosphorus than the uninvolved tissues, and thus be subjected to a selective irradiation from the accumulated radio-phosphorus. More than 100 patients with chronic lymphatic and myelogenous leukemia have undergone therapy with radio-phosphorus during the past three years. Out of this group, 30 patients had received no other form of therapy prior to the use of the radio-phosphorus. One of the patients in this second group has had a complete clinical and hemotological remission for two years, 2 for one year and 16 have had clinical remissions for 6 months

or longer. A sufficient period of time for a thorough evaluation of this new type of therapy has not as yet elapsed, although it appears that more favorable results are to be expected than have been encountered with the conventional methods of treatment of the chronic leukemias. The complete absence of radiation sickness has been consistently observed in all of the patients treated with radio-phosphorus and no other toxic manifestations have been encountered with the therapeutic use of this material. The acute forms of lymphatic and myelogenous leukemia have responded no better to radio-phosphorus than to pre-existing therapeutic methods, such as x-ray, radium, etc. Encouraging results have been noted in several cases of polycythemia vera which have received radio-phosphorus.

Animal experimentation has demonstrated that radio-strontium is deposited in bone with a high degree of selectivity. In view of this observation, radio-strontium has been employed in the treatment of several patients suffering from generalized carcinomatosis which was characterized by widespread metastases in the skeleton. As yet no positive roentgenological evidence of regression of the bony metastases has been observed but some of the patients have had a considerable degree of relief from pain.

Radio-iodine has been employed in the study of the metabolism of iodine by the thyroid glands of normal human controls and patients suffering from several different types of thyroid disorders. It has been observed that, with the aid of radio-iodine, the thyroid glands of these different clinical entities handle iodine in a characteristic manner. It has also been shown that it is possible to completely destroy normal thyroid glands in animals by the administration of radio-iodine without producing demonstrable damage to the other tissues of the body.

*A Study of the Selective Absorption of Iron With the Aid of its Radio-Active Isotope.* By CARL V. MOORE, HAROLD ROBERTS and VIRGINIA MINNICH (introduced by D. P. Barr), St. Louis, Mo.

The work of Hahn, Whipple and their associates has provided convincing proof that iron-deficient dogs absorb larger quantities of iron than do normal animals. While there is considerable evidence that this is also true for man, it has been demonstrated repeatedly, both by balance studies and by the serum iron absorption curve technic, that the normal human adult absorbs relatively large quantities when he is given therapeutic doses of various iron salts. In order to clarify this apparent discrepancy, we have re-studied the absorption of iron both in dogs and in human subjects, using the serum iron absorption technic and the method of Hahn and Whipple which requires the radioactive isotope of iron.

It has been possible to confirm the fact that iron-deficient dogs absorb iron well, while normal animals show only minimal evidence of iron absorption. Normal dogs, however, who have been subjected to one massive hemorrhage seven days before receiving iron, absorb

quantities which closely approximate those absorbed by anemic animals. If an animal whose iron reserves have been exhausted by repeated phlebotomy receives in one massive injection sufficient colloidal ferric hydroxide to replace all of the removed iron, and is studied immediately after the replacement, it is found that he absorbs the metal apparently as well as if there had been no replacement. However, if seven or eight days are permitted to intervene between the time of iron replacement and the absorption study, then only minimal amounts of iron are absorbed. It has been demonstrated that the level of iron in the blood plasma is not the controlling factor which determines the extent of absorption.

In contrast with the studies outlined above, we have continued to obtain evidence that the normal human subject absorbs large quantities of iron when therapeutic doses are given, even though precautions are taken to assure the adequacy of iron reserves.

*The Selective Absorption of Radioactive Iron by Normal and Iron-Deficient Human Subjects.* By JOSEPH F. ROSS and MILAN A. CHAPIN (introduced by James M. Faulkner), Boston, Mass.

Investigations of iron metabolism with the artificially radioactive isotope,  $Fe^{59}$ , have been extended to human subjects. Adults with normal blood and presumably adequate reserves of iron absorbed only very small quantities of the isotope. By contrast, patients with hypochromic anemia and presumably depleted reserves of iron assimilated large amounts of the element.

Negligible amounts of iron were absorbed by patients with untreated pernicious anemia who, presumably, had adequate reserves of iron, even though the anemia was very severe.

Large quantities of iron were absorbed by these same patients, however, after their iron stores had been depleted by producing active hemoglobin regeneration with liver extract therapy, by performing phlebotomies frequently, and by restricting the iron intake. This absorption occurred even though the anemia had abated and was of mild degree.

It would appear, therefore, that the reserves of iron in the body, and not the degree of anemia, determine whether or not iron is absorbed. It is possible that such selective absorption of iron is controlled by the amount of tissue iron present locally in the tissue of the gastro-intestinal mucosa.

*The Effect of Foreign Surfaces on Blood Coagulation.* By EUGENE L. LOZNER and F. H. L. TAYLOR (introduced by George R. Minot), Boston, Mass.

The effects on blood coagulation of glass, paraffin, collodion and a plastic (Lusteroid) were investigated. The platelet content of normal citrated plasma was varied by centrifuging at different speeds and the plasma was then placed in tubes of the four materials. Both the coagulation time of the plasmas on recalcification, and the clot-promoting activity of the plasmas for hemophilic blood, were found to be essentially independent of the platelet

count. Of the various foreign surfaces investigated, glass appeared to exercise the greatest effect on shortening the coagulation time when the plasma was recalcified. The clot-promoting activity for hemophilic blood of normal plasma protected from a glass interface, and of the acid-precipitated globulin fraction derived from such plasma, was considerably less than that of similar preparations exposed to glass. It is concluded that certain foreign surfaces modify blood coagulation by their effects on the factor in normal cell-free plasma concerned with its clot promoting activity for hemophilic blood rather than by their effects on platelet destruction. The implications of these findings to the blood coagulation reaction are discussed.

*Metabolic Studies of the Utilization of Extra Dextrose by Patients with Controlled Diabetes Mellitus.* By JAMES A. GREENE and (by invitation) ANN DAVID, Iowa City, Ia.

Patients with controlled diabetes mellitus have been shown to utilize extra dextrose without an increase of insulin dosage. The mechanism of this utilization has been studied in 8 patients. There have been 27 observations of 4-hour periods in a respiratory chamber during which the gaseous and nitrogen metabolism has been studied. The utilization of the dextrose of the diet was determined first. A few days later the utilization of the extra dextrose was ascertained following the ingestion of the diet plus 50 grams of dextrose. In 4 cases the extra dextrose was administered for from 4 to 6 days, when the second study was repeated. In 4 other cases the utilization of the dextrose equivalent of the diet was also ascertained and a few days later the utilization of the dextrose equivalent of the diet plus 50 grams of extra dextrose was determined. Very little of the extra dextrose was oxidized. Comparison of the non-protein respiratory quotients indicates that it was stored as glycogen. Only one respiratory quotient was above 1.00. A mild insulin reaction in 2 cases reduced the dextrose oxidized.

The significance of these observations upon the treatment of diabetes mellitus is discussed.

*The Influence of Insulin on the Respiration of Human Diabetic Muscle in Vitro.* By HENRY T. RICKETTS and F. J. STARE (introduced by C. Phillip Miller), Chicago, Ill.

The respiration of human diabetic muscle was studied by the Warburg technic in a glucose Ringer phosphate buffer with and without the addition of insulin. In muscle taken from patients with severe, poorly controlled, "insulin sensitive" diabetes, insulin alone, or in one case only after the addition of fumarate, caused an increase of about 40 per cent in the respiration. This effect was not obtained in the muscle of "insulin resistant" diabetics (including two patients with coexisting acromegaly) or of non-diabetic individuals; nor was it observed in any case when pyruvate was substituted for glucose in the buffer.



In the case of one patient with severe diabetes, the oxygen uptake of the muscle removed prior to any treatment was definitely increased by the addition of insulin to the Warburg chamber, whereas no augmentation occurred in tissue obtained after the disease had been adequately controlled with insulin or in a third experiment performed several months later when the patient had become highly resistant to insulin, requiring from 500 to 3000 units per day.

This is the first direct evidence of which we are aware that insulin has an effect on the respiration of human diabetic muscle.

*Factors Influencing Renal Excretion of Calcium in the Normal and in the Hyperthyroid State.* By T. L. ALTHAUSEN and (by invitation) M. STOCKHOLM and W. J. KERR, San Francisco, Calif.

Urinary excretion of calcium was studied in rats from the following aspects: (1) the effect of unrestricted, decreased, and increased water intake; (2) the influence of variations in dietary calcium; and (3) the effect of variations in diuresis produced by xanthin diuretics. These experiments were performed in normal and in hyperthyroid rats.

Our data show that in normal rats the urinary excretion of calcium is directly proportional to the intake of water, to the dietary calcium, and to the degree of diuresis.

Administration of thyroxin resulted in the customary rise of urinary calcium which paradoxically takes place in hyperthyroidism without hypercalcemia. Since our hyperthyroid rats approximately doubled their water intake and ingestion of food (thereby receiving more calcium), and since thyroxin produces diuresis, it appears that these three factors explain the increased urinary calcium output in hyperthyroidism. Conversely, a decrease in water intake or in dietary calcium diminished the urinary calcium of hyperthyroid rats to normal levels, thus further supporting this conclusion.

The bearing of this work and of our previous study of the calcium exchange of the intestine on various hypotheses explaining the negative calcium balance in hyperthyroidism is discussed. Our studies suggest that therapeutic administration of calcium should restore the calcium balance to normal.

*A Renal Concentration Test Employing Posterior Pituitary Extracts.* By WM. A. SODEMAN and (by invitation) H. T. ENGELHARDT, New Orleans, La.

We have abolished the long periods of water deprivation necessary in renal concentration tests by the use of the antidiuretic action of posterior pituitary extracts. Unprepared patients were given 0.5 cc. (10 units) surgical pituitrin and specimens collected at half-hour intervals. Specific gravity was determined with a pycnometer. This procedure was compared with a standard concentration test (modified Fishberg test), the standard concentration test plus pituitary extract, and with the administration of 1600 cc. of water with and without pituitary extract.

In 97 tests on 30 individuals results have been consistent. Posterior pituitary extract in unprepared patients invariably elevated the specific gravity to values which equalled or exceeded those obtained with water deprivation. Water diuresis (1600 cc.) was inhibited by the pituitary extract with maintenance of low urinary volume and elevation of the urinary specific gravity. Results were similar in both normal and abnormal subjects.

Pregnancy and angina pectoris are contraindications to the test. Hypertension is not. The dosage of pituitary extract used has not influenced the blood pressure.

This test eliminates undesirable and unnecessary periods of water restriction. It may be used at any time of day on unprepared patients.

*Renal Blood Flow, Filtration Rate and Tubular Excretory Mass in Patients with Specific Toxemia of Pregnancy.* By CATHERINE A. WELSH, IRWIN WELLEN and HOWARD C. TAYLOR, JR. (introduced by James A. Shannon), New York, N. Y.

This report concerns the measurement of renal blood flow (diodrast clearance), filtration rate (inulin clearance), tubular excretory mass (diodrast  $T_m$ ) and phenol red clearance in patients with specific toxemia of pregnancy. These observations were made on thirteen patients in fourteen pregnancies, both antepartum and postpartum, and in most instances on several occasions.

The patients were seen early in pregnancy when they exhibited no evidence of vascular or renal disease and no hypertension. In the last trimester, hypertension, proteinuria and edema appeared in all. In seven, there was clinical cure after delivery, while in six, hypertension has persisted.

We have studied eight normal pregnant women by the same methods. The results show that there is no change in renal blood flow or filtration rate in normal pregnancy.

Before delivery the patients with specific toxemia showed a slight reduction in filtration rate, a renal blood flow of normal value or distinctly above normal in some instances, and a somewhat reduced filtration fraction.

After delivery, the filtration fraction increased in all; it became normal in the cured group and it rose to above normal in the hypertensive group, in part because of an increase in filtration rate, and in part because of a decrease in blood flow. The fall in blood flow was greater in the hypertensive group. The postpartum findings in the cured group are within normal limits, while the postpartum findings in the hypertensive group present the picture of efferent arteriolar spasm and renal ischemia such as that typically seen in essential hypertension.

The postpartum fall in renal blood flow and increase in filtration fraction were found in the first observation after delivery, regardless of whether the blood pressure had fallen to normal or not. This demonstrates that renal blood flow in these individuals is not determined solely by mean arterial pressure and must be related to renal vascular changes.

In respect to the genesis of hypertension in both the cured and hypertensive groups, it may be emphasized that

these groups present a picture of sufficiency of renal blood flow, or actual renal hyperemia. Thus we do not believe that renal ischemia is primarily responsible for hypertension in toxemia of pregnancy.

*A Comparison of Pituitrin and the Antidiuretic Substance in Human Urine and Placenta.* By GEORGE C. HAM\* (introduced by Eugene M. Landis), Charlottesville, Va.

The presence of an antidiuretic substance in the urine of patients with the toxemias of pregnancy (Teel and Reid, *Endocrinology*, 1939, 24, 297) has been confirmed. On the basis of Gilman and Goodman's results in rats (*J. Physiol.*, 1937, 90, 113), it has been assumed widely that this substance has its origin in the pituitary, but Walker (*Am. J. Physiol.*, 1939, 127, 519) and Arnold (*Arch. f. Exper. Path. u. Pharmacol.*, 1938, 190, 360) found that hypophysectomy did not affect the antidiuretic activity of the urine in animals.

Because of this uncertainty, the antidiuretic substance of human and rat urine has been compared with commercial pituitrin and pituitary extracts with respect to dialysis through cellophane, ultracentrifugation, and urinary chloride excretion. Notable differences between pituitrin and the antidiuretic substance of urine were observed. Placental extracts were found to have antidiuretic activity which closely resembled that of the urine concentrates from the same patients.

The rat method of Burn (*Quart. J. Pharm. and Pharmacol.*, 1931, 4, 517) was used with certain modifications. With uniform water loads, by gavage, the solutions to be tested were injected intraperitoneally and the urine volumes of control and experimental groups were plotted against time. The areas of these curves were measured with a planimeter and the grade of antidiuresis was expressed in square inches.

*Ultracentrifugation.* Samples of potent human urines were ultracentrifuged at 60,000 r.p.m. for 3 to 6 hours, divided into four fractions, and assayed for antidiuretic activity. In every case the fourth or bottom fraction showed the greatest activity. Conversely, varying dilutions of pituitrin solutions ultracentrifuged in the same manner showed no constant difference between the four fractions. The active principle of placental extracts, like the urine substance, was concentrated by ultracentrifugation.

*Dialysis.* The urine and placental antidiuretic factors are not dialyzable through cellophane, whereas the pituitrin factor is dialyzable.

*Chloride excretion.* It is well known that pituitrin increases the urinary chloride excretion and this was confirmed. The antidiuretic substance in urine and placenta did not increase urinary chloride excretion.

*Crude extract.* Commercial pituitrin is a hydrolytic product of the posterior pituitary gland and may therefore consist of a smaller molecule than the native hormone, and thus fail to concentrate in the ultracentrifuge. Crude, saline extracts of fresh, frozen lobes of the posterior pituitary gland were found to be definitely anti-

diuretic and to increase urinary chloride excretion markedly. The antidiuretic factor and the factor which increases urinary chloride excretion were both dialyzable through cellophane and they were not concentrated by the ultracentrifuge. Further work with absolutely fresh press-juice is under way.

*Kidney extracts.* Preliminary work shows that certain concentrations of heated kidney extract produce moderate antidiuresis, while other concentrations are conspicuously diuretic. Both weak and concentrated solutions increase the excretion of chlorides markedly, presumably due to their renin content. Further study of these two effects is being carried on.

### Conclusions

1. The antidiuretic substance in human and rat urine has different physical and biological properties from the antidiuretic factor of pituitrin.
2. The antidiuretic factor in placental extracts has in three respects, at least, the same physical and biological properties as the urine factor.

*Prostate and Serum "Acid" Phosphatase.* By ALEXANDER B. GUTMAN and (by invitation) ETHEL B. GUTMAN, New York, N. Y.

Further studies have been made of (1) the physiological significance of the specific "acid" phosphatase present in high concentration in human prostate tissue, (2) of the usefulness of serum "acid" phosphatase determinations in the diagnosis of metastasizing prostate carcinoma.

It has been found that "acid" phosphatase first appears in the prostate gland at puberty, either normally or artificially induced; that the enzyme content of seminal fluid is extremely high, relatively constant for any one individual, and largely contained in the first cc. of ejaculate; that the enzyme may form part of an extracellular glycolytic system (analogous to intracellular erythrocyte phosphatase), participating in the largely glycolytic metabolism of human spermatozoa.

Invading prostate carcinomatous tissue secretes "acid" phosphatase into the circulating fluids where the enzyme can be determined and the primary tumor identified. Our experience with 62 cases of metastasizing prostate carcinoma, in 37 cases of prostate carcinoma without demonstrable metastases and in several hundred miscellaneous cases indicates that the serum "acid" phosphatase determination is useful in detecting metastasizing prostate carcinoma, and occasionally before this is possible by x-rays. Paget's disease and other osteoplastic bone lesions can be differentiated.

*Nitrogen Balance in Cirrhosis of the Liver.* By JOSEPH POST (by invitation) and ARTHUR J. PATEK, JR., New York, N. Y.

Nitrogen balance studies were performed on 5 patients with cirrhosis of the liver, ascites, and hypoalbuminemia in order to determine whether the hypoalbuminemia was due to altered protein synthesis or to protein starvation caused either by low protein intake or by faulty assimila-

\* Commonwealth Fund Fellow.

tion. Abdominal paracenteses were not done during the study periods. In 3 patients the nitrogen balance was determined for 48-hour periods at 2-week intervals for 1, 2, and 2 months, respectively. Two other patients were studied for 25 and 32 consecutive days. The patients were fed daily 1.5 to 2.0 grams of protein per kilo body weight.

The results show that the fecal nitrogen was within normal limits. All patients were in positive nitrogen balance. No correlated rise of serum albumin was associated with this nitrogen retention. The serum globulin and non-protein nitrogen were unchanged. This type of balance pattern differs from that seen in nutritional hypoproteinemia, in which a rapid rise of serum albumin follows protein feeding. The findings indicate that patients with cirrhosis of the liver, ascites, and hypoalbuminemia can absorb and retain nitrogen from the food protein. However, there appears to be a defect in the synthesis of serum albumin.

*Dietary Liver Disease in Rats.* By GRAHAM WEBSTER (introduced by Joseph T. Wearn), Cleveland, Ohio.

Rats given a diet of 8 per cent casein, 38 per cent fat, 2 per cent cod liver oil, 5 per cent salts and 47 per cent rice starch with 20 gamma each of thiamin chloride and riboflavin or Brewer's yeast  $\frac{1}{2}$  gram q.o.d. developed a disease of the liver consisting of portal cirrhosis with occasional necrosis at the end of a 150-day period. They also exhibited nephrosis and cortical necrosis of the kidneys. These lesions could be prevented by increasing casein to 18 per cent, by the addition of betaine 50 mgm. q.d., and by diminishing fat. The addition of cystine 5 mgm. q.d. and of cholesterol 2 per cent aggravated the disease and could be inhibited by the addition of betaine. Whole yeast 1 gram daily or molasses 2 grams daily, added to the original diet in place of some carbohydrate, prevented the lesions. Male rats of more than 250 gram weight were most susceptible to liver changes. Kidney changes occurred at any age. In the rats receiving added cystine there was an incidence of neoplasms in 20 per cent; 2 hepatomas, 1 carcinoma of the lung, 1 adenocarcinoma of the pancreas and 1 unidentified retroperitoneal tumor. No tumors occurred in any of the other rats used in the experiment.

*Studies on Blood Flow in the Gastro-intestinal Tract of Man.* By C. H. RICHARDS and STEWART WOLF (by invitation) and H. G. WOLFF, New York, N. Y.

A method of measuring and recording changes in the blood flow of the intestinal mucosa has been developed. The essential element consists of a silver button, which carries a heater and a thermocouple, mounted on the surface of a balloon. The button is applied to the mucosa of the gut by inflating the balloon, and the temperature of the button is raised about 2° C. above body temperature by connecting the heater to a suitable D.C. source. The reference thermocouple is maintained at body temperature. With constant heat supplied, the temperature of the button will be a function of the rate at which heat is conducted away by the blood flowing past it.

Blood flow (temperature) is recorded by connecting the thermocouples to a galvanometer, the light reflected from its mirror varying the illumination of a photoelectric cell. The current from this cell is then amplified and fed into a solenoid whose movable core carries a writing point.

Pressure is recorded simultaneously by a similar solenoid and amplifier controlled by the height of an Hg column in contact with a high resistance wire.

It has been shown by this method that contractions in the duodenum, ileum and colon are usually associated with a transient increase in mucosal blood flow. In fasting subjects the sight and smell of food were accompanied by a marked increase in flow in the duodenal mucosa. Experimentally induced emotional states, e.g., anxiety and resentment, may also augment the circulation in the duodenum.

*On the Mechanism of Insulin Resistance in Toxemic States.* By MATTHEW TAUBENHAUS (by invitation) and SAMUEL SOSKIN, Chicago, Ill.

Previous work has clearly shown that the liver is a major factor in determining carbohydrate tolerance and the reaction of the whole animal to insulin. The study of the hepatic enzyme systems involved in these processes, and of the influence of toxin upon them, necessitates the frequent sampling of the liver for biological assay. The present work was therefore done on normal dogs and on animals treated with diphtheria toxin.

It is known that hepatic glycogenolysis normally proceeds by phosphorylation, and that insulin acts upon this system, although the exact point of action is still in doubt. The present work shows that toxic hepatic damage inhibits the phosphorylating systems, and at the same time allows the blood amylase, which is normally excluded from (or inactive within) the liver cell, to enter and break down glycogen. Insulin does not influence this latter abnormal pathway of glycogenolysis and hence an abnormally small response to insulin is exhibited. This work also supplies a rationale for high carbohydrate therapy in insulin resistance on the basis of mass action on the amylolytic enzymes.

*The Excretion of Zinc Uroporphyrin in Idiopathic Porphyrria.* By C. J. WATSON and (by invitation) SAMUEL SCHWARTZ.

Five cases of idiopathic porphyria, either abdominal, nervous, or mixed in type, have been studied during the past three years. Three of the five cases died and were examined at necropsy; two are living and have improved to some extent.

All of the cases have been characterized by the excretion of uroporphyrin in both urine and feces in the form of the zinc complex. The occurrence of uroporphyrin in the feces has hitherto not been described. The excretion of uroporphyrin as the zinc complex in five successive cases of this disease indicates that zinc uroporphyrin is a disease entity, and that excretion of uroporphyrin as the zinc complex is the usual rather than the unusual mode of elimination in so-called "acute" idio-

pathic porphyria. The site of formation of uroporphyrin and the significance of its combination with zinc have not been determined.

Except in one instance, neither free uroporphyrin nor its zinc complex has been found in the bile, gall stones, or duodenal contents. Zinc uroporphyrin was identified with certainty in the duodenal contents of one of the cases. The amount appeared to be relatively small in comparison with that noted in the urine and feces of the same case. The significance of this absence or relative lack of uroporphyrin in the bile, as compared with the urine and feces, is not yet clear. Two possibilities may be considered: (1) that the substance is formed in the bowel and deposited in part in the liver (where it has been found in large amounts at necropsy), another part passing into the general circulation and thence into the urine; (2) that the substance is formed in the body and excreted through the intestinal mucosa. This possibility is considered chiefly because of the known intestinal excretion of zinc.

In addition to the zinc complex of uroporphyrin, the urines of these patients regularly exhibited positive Ehrlich reactions, due chiefly to porphobilinogen. A simple method serving to distinguish this substance from urobilinogen is described. This depends upon chloroform solubility. The Ehrlich compound of urobilinogen is readily extracted with chloroform while that of porphobilinogen is not.

*Physiological Effects of Reduction of Internal Temperature of the Body.* By JOHN H. TALBOTT, Boston, Mass.

A marked reduction of the internal temperature of the body was achieved by the technique of Smith and Fay. Thirty-five experimental periods were studied in a group of 15 persons under 40 years of age. All were suffering from severe mental disorders but were essentially normal otherwise insofar as could be determined by clinical examination of the internal organs. The experimental periods varied from 24 to 72 hours. Internal temperatures as low as 75° F. were observed. Restoration of body temperature was uneventful and no serious complications or consequences were noted. Extensive physiological, biochemical, and clinical data were collected during each low temperature period. These concern (1) respiratory function of the blood, (2) carbohydrate metabolism and (3) cardio-vascular-renal function.

Changes in the third category are discussed in this communication. The size of the heart, as measured by the teloröntgenogram, decreased slightly. Cardiac arrhythmias appeared when the rectal temperature fell below 85° F. These and other variations were recorded on the electrocardiograph. A pronounced constriction of the arteries, veins and capillaries of the periphery appeared early in each experimental period and persisted. The peripheral blood pressure was frequently unobtainable for several hours. The time for blood to pass from arm to lung was increased. Evidence of reduction of circulating blood in the internal organs was obtained from the studies of renal function. Clearance of inulin, creatinine and

diodrast by the kidneys, as well as determination of maximum tubular activity with high concentrations of glucose and diodrast in the blood, was investigated. All measured functions of the kidney were reduced by as much as 50 per cent. An approximate temperature coefficient was demonstrated, i.e., the lower the internal temperature of the body, the greater the reduction in kidney function. Following restoration of body temperature the kidney function was normal.

*The Comparative Effects of Liver and Kidney Disorders on the Proportions of Phosphatide Phosphorus, Free and Ester Cholesterol in Blood Serum.* By EDWIN F. GILDEA and (by invitation) EVELYN B. MAN, New Haven, Conn.

Previously reported investigations, indicating that serum phosphatide phosphorus varies directly with changes in total cholesterol in normal, hyper- and hypothyroid and malnourished subjects, have led to a search for disorders in which this relation might be disrupted.

In 14 of 30 patients with a number of symptoms of severe liver disease, the proportions of phosphatide phosphorus to total cholesterol deviated markedly from the normal, the phosphorus fraction being relatively high. As the liver disease progressed, both lipid components ultimately decreased to below normal levels. These changes tended to parallel the amount of reduction in esterified cholesterol.

Similar data on 8 patients with nephrosis also revealed a disproportion between phosphatide phosphorus and cholesterol but in the opposite direction from that in the patients with liver disease. When remissions occurred in the nephrosis these lipid components were restored to normal proportions.

In 26 patients with chronic nephritis (chiefly glomerular in type in most cases), the amounts of phosphatide phosphorus and cholesterol approximated normal proportions. Five of these patients, however, had abnormally high lipoids with comparatively low phospholipid phosphorus, as in the nephrosis cases. As the nephritis became more severe, the lipoids tended to fall to below normal levels and the lipid components returned to normal proportions.

In addition to demonstrating the intimate association of phosphatide and cholesterol metabolism, the above findings contribute to the already extensive data indicating that the liver plays a fundamental rôle in lipid metabolism, and suggest that the kidneys may take a more important part in controlling blood lipoids than has commonly been attributed to them.

*Pathological Variations in Blood Pyruvic Acid.\** By ERNEST BUEDING, HERMAN WORTIS and MARTIN H. STEIN (by invitation) and NORMAN JOLLIFFE, New York, N. Y.

Coccarboxylase (thiamin pyrophosphate) is necessary for the normal catabolism of pyruvic acid. We have deter-

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Cocarboxylase (thiamin pyrophosphate) is necessary for the normal catabolism of pyruvic acid. We have deter-

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mined blood pyruvic acid levels in 280 individuals and have obtained the following results:

1. In 39 normal adults, the fasting pyruvic acid level in the blood ranged from 0.77 to 1.23 mgm. per 100 cc., the average being 1.00.

2. In 79 individuals with "functional" psychoses and psychoneuroses, the values ranged from 0.71 to 1.29 mgm. per 100 cc., the average being 0.98.

3. In 35 patients with organic neurological disease without evidence of nutritional deficiency, the values ranged from 0.63 to 1.17 mgm. per 100 cc., the average being 0.97.

4. In 56 patients with various medical disorders not complicated by fever or nutritional deficiency, the values ranged from 0.49 to 1.31 mgm. per 100 cc., the average being 0.94.

5. In 33 alcohol addicts who showed no evidence of acute peripheral neuropathy, the values ranged from 0.54 to 1.12 mgm. per 100 cc., the average being 0.92.

6. In 38 cases associated with thiamin deficiency (acute peripheral neuropathy in alcoholics, Wernicke's syndrome and beriberi heart disease), the values ranged from 1.43 to 3.63 mgm. per 100 cc., averaging 1.92.

It has also been demonstrated that, following the ingestion of glucose (1.75 mgm. per kgm.), there is a rise in blood pyruvic acid, and in normal subjects the curve follows a fairly characteristic pattern, generally returning to normal in three hours.

Finally, evidence is presented to show that, in cases associated with thiamin deficiency, the pyruvic acid curve following glucose ingestion is abnormally elevated, and fails to return to the fasting level. Following therapy with thiamin, the fasting pyruvic acid level and the pyruvic acid curve return to normal.

*The Effects of Tyrosinase on Arterial Hypertension.* By HENRY A. SCHROEDER and MARK H. ADAMS (by invitation) and ALFRED E. COHN, New York, N. Y.

An attempt has been made to learn something of the nature of the pressor substance or substances believed to be responsible for arterial hypertension. According to Holtz, decarboxylation but not deamination of certain amino acids occurs in the ischemic kidney, leading to the formation of pressor amines. As the most powerful pressor amines are phenols, tyrosinase was used. This phenolic oxidase was found to lower the blood pressure of rats and dogs made hypertensive as the result of experimental renal ischemia. It was therefore injected into human beings. Twenty patients exhibiting arterial hypertension of various stages were given daily subcutaneous injections of this enzyme. In one there was little effect; in three the blood pressure fell to slightly lower levels. In the remainder it appeared that the disease temporarily was definitely altered. This was accompanied by changes in the level of blood pressure, regression of hemorrhagic and exudative lesions in the ocular fundi (eleven cases), diminution in the size of the heart in x-ray photographs (five cases), diminution in the level of the urea nitrogen in the blood without change in urea clearance (nine

cases), increase in the clearance of urea (six cases) and disappearance of symptoms, such as headache (ten cases), palpitation (seven cases), dyspnea and orthopnea (two cases) and edema (one case). In nine, electrocardiograms were altered in the direction of normal.

This enzyme, a protein, acts as an antigen. In four individuals severe allergic reactions developed at the site of injection. Changes in the level of blood pressure occurred before these appeared.

When injections were stopped, the blood pressure soon (within three to seven days) returned to its previous level. Subsequent series of injections were given to three patients with similar results. Because this enzyme is specific for phenolic compounds, it is believed that a phenolic substance common to some hypertensive states is altered. It is not yet known that this phenol is the pressor substance but the fact that the pressor action of angiotonin is directly inactivated by tyrosinase suggests that hypothesis.

*The Nature of the Arterial Hypertension Produced in Normal Subjects by the Administration of Angiotonin.*

By ROBERT W. WILKINS and (by invitation) CHARLES N. DUNCAN, Boston, Mass.

Angiotonin administered intravenously in single doses or by continuous infusion to normal subjects caused increases of systolic and diastolic arterial pressure, rises of venous pressure, and decreases of vital capacity proportional to the dosage used. During the hypertension there was bradycardia, and a decrease in the stroke volume of the heart, resulting in a striking reduction of cardiac output (ballistocardiographic). Teleoroentgenogram revealed usually a small increase in heart size, never a decrease. In the limbs there was often a decrease in blood flow as measured plethysmographically or by skin temperature, but usually within the normal range. Blood flow during reactive hyperemia (after 5 minutes' arterial occlusion) in the muscular segments increased with the rise of arterial pressure. Sympathetic vasomotor reactions were retained, and the pressor response to the cold test of Hines and Brown was not altered. Circulation time often increased, but remained within normal limits. Spinal fluid pressure was not significantly changed. Mild symptoms of dizziness, substernal oppression, nausea or palpitation were occasionally noted. The effects continued at a stable level during a constant infusion, and they subsided 4 to 8 minutes after the cessation of the administration, whether infusion or single injection.

*Unilateral Renal Function in Essential Hypertension.* By HERBERT CHASIS and JULES REDISH (introduced by William S. Tillett), New York, N. Y.

The clearance method has been used in unilateral studies in order to obtain evidence on the question as to whether unilateral renal ischemia is present in patients with arterial hypertension.

Twenty patients with essential hypertension have been studied. The observation started with a cystoscopic examination, after which ureteral catheters were inserted



for a distance of 12 cm. The cystoscope was then removed and a urethral catheter was placed in the bladder in order to detect extra-catheter leakage. Urine and blood samples were collected in the usual manner of the clearance technique. Effective renal blood flow, filtration rate and tubular excretory mass were measured in the separate kidneys. Finally, retrograde pyelography was done in the supine and sitting positions.

Diodrast clearance and  $T_m$  were measured in 10 patients, using both ureteral and bladder catheter techniques. The results were in agreement. The unilateral methods also gave results in normal individuals which were comparable to those obtained in a large series of normals studied by the bilateral technique.

The absolute rate of glomerular filtration was usually reduced in hypertensive subjects. This has been interpreted as indicating obliteration of glomeruli in both kidneys.

The tubular excretory mass was reduced in every hypertensive subject studied. These findings are interpreted as indicating a progressive destruction of renal tubular tissue.

If those patients in whom renal hyperemia is present as a result of either increased glomerular pressure or impotent tubules are omitted, the blood flow per unit of tubular mass was found to be subnormal in every kidney.

This renal ischemia is primarily caused by efferent arteriolar constriction, since the reduction in blood flow is associated with an increase in the filtration fraction.

The specific gravity of urine simultaneously collected from either kidney usually is similar, but occasionally the kidneys excrete different concentrations of urine at the same time, in spite of an equal blood flow, filtration rate and tubular mass. Observed inequality in urine flow is important in that excretory tests of renal function can readily be misinterpreted. Urinary dye concentrations may vary from the two kidneys and yet their blood flow and functional capacity may be equal. This point is also significant in intravenous pyelography, since differences in concentrations of diodrast referable solely to differences in urine flow may result in differences in densities of the x-ray shadows.

Abnormal pyelographic findings are not necessarily associated with functional changes, and marked functional disparity in the two kidneys may not be associated with abnormal retrograde pyelograms. Many of the variations in uretero-pyelograms are believed to be without significance.

As the destruction of the renal parenchyma progresses in hypertensive disease, the blood flow per unit of tubular excretory mass remains equal in the two kidneys. In no instance in this study was there any indication of a unilateral ischemic kidney.

*Adenomatous Hyperplasia of the Adrenal Cortex in Essential Hypertension.* By JAMES F. RINEHART and (by invitation) O. O. WILLIAMS and WILLIAM S. CAPPELLER, San Francisco, Calif.

Adrenal glands removed routinely at necropsy have been carefully weighed and studied. In a series of 26

cases of essential hypertension, the mean weight of the glands has been found to be approximately one-third greater than in a control group of 100 non-hypertensive cases. The enlargement is due to a nodular or adenomatous hyperplasia of the cortex. This is frequently quite striking and is found to some extent in practically all cases. Microscopically the cortical cells are arranged in irregular, tortuous columns. The cells are hyperplastic and show a high lipid content. Changes of this type are infrequently seen in non-hypertensive cases. One of 4 cases of glomerular nephritis and 2 of 9 cases of chronic pyelonephritis showed adenomatous hyperplasia of the adrenal cortex comparable to that seen in essential hypertension and 8 of the cases of chronic pyelonephritis showed more than the average amount of lipid in the cortical cells. This suggests that some stimulation of the adrenal cortex may result from a renal type of hypertension. While the adrenal cortical hyperplasia which we have shown to occur commonly in essential hypertension may also be a secondary effect, it is suggested that a primary hyperplasia and hypersecretion of the adrenal cortex might induce hypertension. In view of the anatomic evidence presented and other data cited, this possibility seems worthy of further investigation.

*Factors Causing Increase in Venous Pressure of the Lower Extremities During Abdominal Operations.* By DAVID DAVIS and (by invitation) SAMUEL GILMAN, Boston, Mass.

The venous pressure in the lower extremities has been studied during and following abdominal and pelvic surgery. Four factors produced appreciable increases in venous pressure: (1) packing or traction of certain structures such as the uterus or gall bladder; (2) the Trendelenburg position; (3) the abdominal binder; and (4) postoperative abdominal distention.

During 7 operations on the uterus, 5 of which were for hysterectomy, increases in venous pressure above 40 cm. of water were maintained for one-half to one and one-half hours; in 5 operations there were transient rises above 60 cm. and in two, such rises were maintained for 18 to 33 minutes.

These increases were partly due to the Trendelenburg position. It was found that this position alone caused increases in venous pressure proportional to the angle of the table. In one case, for example, as the head of the table was dropped 10 degrees, venous pressure increased 15.5 cm.; at an angle of 20 degrees, 34.0 cm.; and at an angle of 30 degrees, 49.0 cm. of water above the horizontal level. These increases generally persisted as long as the Trendelenburg position was maintained. Studies with Dr. A. S. Freedberg showed that the femoral-to-carotid sinus circulation time in dogs is considerably slowed with the assumption of the Trendelenburg position.

In 2 of 3 operations for cholecystectomy less striking but appreciable rises in venous pressure were noted during packing or traction on certain structures. Few changes, on the other hand, were observed in the course



of 5 operations for unilateral inguinal hernia. The application of the binder in 3 of these, however, produced an immediate and significant rise in venous pressure; in one an increase of 12 cm. persisted during the 30-minute period of postoperative observation. In 2 patients with postoperative distention the femoral venous pressures were elevated.

With marked and at times prolonged increases in venous pressure the possibility of injury to the venous bed and subsequent thrombophlebitis must be entertained. Furthermore, factors causing increases in venous pressure either as a result of mechanical obstruction or irritation leading to contraction of veins may continue to operate in the post-operative course. A tight abdominal binder may retard venous circulation, and this is possibly true of a loose one when abdominal distention develops.

*Demonstration that the Cell Plasma Ratio of Blood Contained in Minute Vessels is Lower than That of Venous Blood.* By EUGENE A. STEAD, JR. and (by invitation) RICHARD V. EBERT, Boston, Mass.

Direct observation of the minute blood vessels has shown that the red cells flow in the rapidly moving central portion of the stream and that there is a slow moving clear layer of plasma adjacent to the wall of the vessel. Because of the size of this peripheral layer of clear plasma in the minute blood vessels, it has been suggested that the cell plasma ratio of blood contained in the minute vessels is lower than that of blood from the large vessels.

The cell plasma ratio of the blood normally contained within the minute vessels has not been previously determined because of the difficulty in obtaining a sample of this blood. Blood collected from a vein or from the cut ends of minute vessels is representative of the blood flowing *from* the minute vessels but it is not necessarily representative of the blood contained *within* the minute vessels. The blood contained within the minute vessels consists not only of the central core of red cells and plasma which is flowing rapidly into the veins, but also of the slower moving peripheral layer of plasma.

A portion of the blood normally contained within the minute vessels was obtained by the following technique: (1) the arterial inflow to the arm was obstructed; (2) the blood from the larger vessels was removed by needle and syringe; (3) the blood normally contained within the minute vessels was then forced into the veins of the forearm by applying an Esmarch's bandage.

In 15 experiments the hemoglobin concentration of the blood milked from the minute vessels was from 0.8 to 1.8 grams lower than the hemoglobin concentration of venous blood. Determinations of serum protein concentration and of hematocrit reading showed that the lower hemoglobin concentration of blood normally contained within the minute vessels could not be accounted for by the entrance of extracellular fluid into the blood stream or by a shift of fluid from red cells to plasma.

The following conclusions have been drawn:

1. The venous blood is richer in cells and poorer in plasma than the blood contained within the small vessels.

Therefore, the cell plasma ratio of blood drawn from artery, vein, or finger is not representative of the cell plasma ratio of the entire circulating blood.

2. The value for the red cell volume as calculated from the plasma volume and hematocrit reading is falsely high because of the uneven distribution of cells. The value for the total circulating hemoglobin, as calculated on the basis of the plasma volume, hematocrit reading and hemoglobin concentration is also falsely high.

3. It is not possible to quantitate accurately changes in plasma volume from the changes in hematocrit reading or hemoglobin concentration.

*Pituitary-Diabetes in the Cat Treated by Low Diet, Insulin, Phlorhizin and Adrenalectomy.* By F. D. W. LUKENS and (by invitation) F. C. DOHAN, Philadelphia, Pa.

Pituitary-diabetes in the cat provides a form of stable diabetes with a reversible island lesion (hydropic degeneration for the first 3 months) in which functional recovery of the animal and morphological restoration of the islands may be studied. Partially depancreatized cats which had recovered from the operation without diabetes were injected with crude saline anterior pituitary extract until they remained diabetic after the extract was discontinued. We have described the recovery following insulin treatment in such animals. This recovery was independent of the severity of the disease, but was limited by the duration of the diabetes and the ultimate development of irreversible lesions (atrophy and fibrosis). Results of methods other than treatment with insulin are now presented.

Reduction in diet for 24 to 26 days was followed by recovery in two cats with mild diabetes. This means that on resuming their original diet they gained weight and the blood sugar remained normal. Five cats with moderately severe diabetes were not controlled by a similar reduction in diet. Unlike insulin, the result from dietary treatment is greatly limited by the severity of the disease.

Diabetic animals recovered following the administration of phlorhizin for 2 to 3 weeks. Restoration of the islands also followed adrenalectomy. After adrenalectomy the extent of functional recovery was uncertain. The four procedures (low diet, insulin, phlorhizin, adrenalectomy) have in common a favorable influence in this type of diabetes, and all reduce the blood sugar to normal levels during treatment. As they differ in many other respects these methods should afford a new approach to the physiological analysis of this disease.

*The Relation of Magnesium to the Thyroid Hormone.* By PAUL H. LAVIETES and (by invitation) ROBERT F. DINE, New Haven, Conn.

The observation of Soffer and his collaborators that non-ultrafiltrable, or bound, magnesium is consistently elevated in hyperthyroidism and is absent in myxedema has been confirmed. The technique of ultrafiltration and analysis has been modified. Bound magnesium remains above normal after treatment of hyperthyroidism with

iodine, a matter of considerable importance when diagnosis has been obscured by previous administration of iodine. In 7 patients with hypermetabolism without hyperthyroidism, bound magnesium was uniformly normal. Hyperthyroidism was excluded in these patients by observations of normal serum iodine and failure to respond to iodine therapy. Except for 5 cases of frank myxedema, in which bound magnesium was entirely absent, bound magnesium was observed to be below the lowest normal value, 11.3 per cent, in only 4 instances. Collateral clinical and laboratory evidence is adduced to indicate that these cases may actually represent partial hypothyroidism.

Data relating bound magnesium to iodine in serum suggest that magnesium may be an integral part of the circulating thyroid hormone, or of the complex in which the hormone functions.

*Protein-Bound Iodine in Blood Plasma.* By WILLIAM T. SALTER and (by invitation) A. MERTON BASSETT and ALBERT H. COONS, Boston, Mass.

Although hormone in the thyroid gland occurs as thyroglobulin, the protein-bound iodine in the blood plasma of man and of the horse was found to reside prominently in the traditional albumin fraction. The protein-bound iodine is subject to fluctuations, depending upon thyroid activity. Such fluctuations are due chiefly to the thyroxine-like moiety thereof. Although the fraction resembling diiodotyrosine may vary proportionately, because of its small magnitude it contributes very little to the absolute increment. Despite variations in the protein-bound iodine, the inorganic iodine concentration is rather low and approximately constant. These findings suggest that the protein-bound moiety of plasma iodine may be used as an objective index of circulating thyroid hormone and, indirectly, as a measure of thyroid activity.

A series of 94 cases was analyzed from this standpoint. In about two-thirds of them the clinical diagnosis and the basal metabolic rate were consistent and there was a high correlation between the latter and the protein-bound iodine. Of the remaining one-third, the basal metabolic rate did not clearly reflect the clinical status, whereas the protein-bound iodine was more reliable. In hypothyroidism, plasma protein-bound iodine was consistently low and the thyroxine-like fraction thereof almost nil. Of special interest is the exophthalmic ophthalmoplegia group, classified as "Graves' disease without hyperthyroidism," in which the basal metabolic rate was often within normal limits and the plasma protein-bound iodine was also normal.

*Collection of Iodine in the Thyroid as a Differential Criterion in the Diagnosis of Two Types of Graves' Disease.* By S. HERTZ and A. ROBERTS (introduced by J. H. Means), Boston, Mass.

In another place a rather extended description of a special variety of Graves' disease in which the eye symptomatology is dissociated from the thyrotoxic element is described by Hertz, Means and Williams. We wish to present here data bearing on the difference in iodine

metabolism in the two types of Graves' disease, as determined by the use of radioactive iodine as a tracer in the study of thyroid physiology. The pattern of collection of the iodine in the thyroid in ordinary Graves' disease follows a definite curve; the collection in the thyroid of the special variety has a differently shaped curve; and both of these are quite separable from the curve for normal patients. In general, the method used was as follows: one milligram of labelled iodine was administered by mouth, and the iodine uptake in the gland was measured at various time intervals by means of a Geiger-Müller counter externally placed over the thyroid.

*The Effect of Aluminum Hydroxide Ingestion on the Phosphorus and Calcium Disorders of Hypoparathyroidism.* By FULLER ALBRIGHT and (by invitation) CHARLES H. BURNETT, WILLIAM PARSON, and HIRSCH W. SULKOWITCH, Boston, Mass.

Aluminum hydroxide has long been used in the production of experimental rickets in animals. It produces its effect by uniting with phosphates in the gastro-intestinal tract and preventing their absorption. Inasmuch as it has for several years been the opinion of those in this laboratory that the disorder of calcium metabolism in hypoparathyroidism is dependent on a more fundamental disorder in phosphate metabolism, it seemed of interest first to determine whether the administration of aluminum hydroxide would lower the high serum phosphorus level in hypoparathyroidism by preventing phosphate absorption and, secondly, whether it would elevate the low serum calcium value. Such was found to be the case, although the results were not quite those that would have been predicted. The studies include complete metabolic data on one patient.

*Replacement of Potassium by Sodium in Muscles of Normal Dogs Receiving Desoxycorticosterone Acetate.* By JOSEPH W. FERREBEE, DONALD PARKER, WILLIAM H. CARNES, and MILDRED K. GERITY (by invitation) and DANA W. ATCHLEY and ROBERT F. LOEB, New York, N. Y.

Normal dogs receiving daily subcutaneous injections of 25 milligrams of desoxycorticosterone acetate develop diabetes insipidus and attacks of profound muscular weakness. In experiments on six normal animals it was found that administration of desoxycorticosterone acetate caused an increase in intracellular sodium and a decrease in intracellular potassium of skeletal muscle, but no change in the extracellular water jacket of the muscle, that is, no change in the so-called chloride space. The changes in intracellular sodium and potassium concentrations could be prevented by the administration of potassium chloride which maintained a normal relationship of sodium to potassium in the cell and prevented the occurrence of paralysis. The diabetes insipidus was independent of the muscle electrolyte pattern and developed whether or not the animals were given potassium chloride. The diabetes insipidus could be correlated with the fact that all the animals receiving hormone had an elevation of serum

sodium concentration. On the other hand, a high serum sodium concentration was not responsible for the increase in muscle sodium. The serum sodium was elevated in the animals on potassium chloride in whom no disturbance of muscle electrolyte concentrations was observed. This latter circumstance offered evidence that sodium goes into muscle cells only when potassium comes out, that is, sodium will replace but not displace potassium. This view is consistent with the idea that desoxycorticosterone, by increasing the renal excretion of potassium, lowers the serum potassium concentration and shifts the equilibrium of potassium between serum and cell in the direction of an increased loss of potassium from the cell.

*The Peripheral Nature of the Muscular Weakness of Familial Periodic Paralysis.* By GEORGE D. GAMMON and A. M. HARVEY, Philadelphia, Pa.

Myograms made during the development of the attack show a gradually rising threshold to electrical stimulation of the motor point, failure of response to single shocks, then to stimuli below 15 per second, and finally complete failure to any frequency of stimulation.

Electromyograms from the short flexor of the fifth finger are of low voltage and of nearly monophasic form in belly-tendon leads, but leads more closely spaced show increasingly diphasic responses. This suggests that the impulse fails to spread throughout the length of the muscle fiber.

Circulatory obstruction of the arm during an attack lowers the threshold and increases tension and voluntary strength. The voltage of the electromyogram increases, and its form becomes more diphasic.

The potassium salts restore strength and reverse the changes described above. At the onset of recovery a tetanus clearly augments the responses, just as exercise will hasten recovery.

In the attack the disturbance involves the muscle fiber and the action potential appears not to be conducted throughout its length. The defect is restored by maneuvers which probably increase the potassium outside the muscle fiber, relative to that inside, by a loss of fiber potassium during exercise or asphyxia or by an increase of serum potassium from administration of the salt.

*Studies in Muscular Tension in the Neuroses.* By JURGEN RUESCH (by invitation) and JACOB E. FINESINGER, Boston, Mass.

Muscular tension was studied in a series of 38 psychoneurotic patients of varied diagnoses and 12 normal control subjects by the use of two methods. The first method made use of a stylus, the point of which was attached to a rubber bulb to record the pressure exerted on the point (point pressure) during writing. The stylus was enclosed in another rubber bulb which was held by the subject during writing to obtain the grip pressure. Each bulb was connected by means of pressure tubing to a Marie tambour. The oscillations of the tambours were photographed on a moving film and in this way records of

the point and grip pressure used during the writing of a standard paragraph could be calibrated and studied. After the records of handwriting and pressure had been made, the subjects were given a questionnaire to determine their mental status with special reference to feelings of general and neuromuscular tension. The second method involved the study of electromyographic tracings obtained from the extensor and flexor forearm muscles of the same group of patients and control subjects while they were asked to make a fist at given time intervals. From the electromyographic tracings the time taken for the motor activity and for relaxation could be measured.

The results obtained indicated the following:

1. The writing time for the patients was considerably greater than that of the control group.
2. The patients showed a greater variation in both point and grip pressure than did the control subjects.
3. Greater values for grip pressure were obtained in the group of patients than in the control group.
4. The group of patients had a much shorter relaxation time between discrete muscular activity (making a fist) than did the group of normal controls.
5. There was a strikingly positive correlation between high values for grip pressure and the presence of neuromuscular tension as reported by the subjects.

*Involuntary Activity in Skeletal Muscle in Joint Disease: Electromyographic Observations.* By CHARLES L. SHORT and (by invitation) ALFRED O. LUDWIG and ROBERT S. SCHWAB, Boston, Mass.

Muscle atrophy is one of the most disabling features of rheumatoid arthritis. The factors responsible have not yet been established, although clinical and experimental evidence suggests a reflex mechanism from impulses set up in the diseased joints.

We have recorded electromyograms from patients with joint disease by means of a Loomis ink-writing oscillograph. While the tracings of voluntary contractions demonstrated no constant deviation from the normal, many of the patients showed action potentials while immobile and apparently relaxed. These usually consisted of a series of regular diphasic spikes at a rate varying from 6 to 15 per second and with an amplitude as high as 40 microvolts. Such patterns were found in 10 of 20 patients with rheumatoid arthritis, as well as in gonorrheal arthritis and joint limitation due to fixation. The activity usually appeared and disappeared spontaneously, might alternate from spot to spot within the muscle, and might be present in one muscle related to a joint and not in another at the same time. In certain cases, the series of spikes could be made to disappear on altering the position of the limb, but in other cases, it persisted, uninfluenced by voluntary and reflex contraction of the muscle.

The nature and origin of this involuntary muscle activity cannot be defined at present, but consideration is given to the possibilities that

1. It arises by a reflex path from impulses set up in the joint.

2. It is a factor in the production of muscle atrophy in joint disease.

#### READ BY TITLE

*The Host Factor in Protection by Vaccine Immune Serum.* By R. F. PARKER and (by invitation) R. H. GREEN, Cleveland, O.

Early experiments gave results which were interpreted as indicating that infection with the virus of vaccinia might follow the suitable introduction of a single viral particle. Later work indicated, however, that while this was true for fully virulent strains, it did not hold when strains of lower virulence were tested, the evidence seeming to indicate that under these conditions it was the chance of entry of the virus into a susceptible cell which determined whether or not infection occurred. In respect to the proportion of cells susceptible, variation between animals was observed. In the protection test with immune serum it has been found that, with increasing amounts of serum, the regularity of the titration curve decreased. The present experiments indicate that this is due primarily to variation between the response of individual animals. It is suggested that the action of immune serum is to render a certain proportion of the host cells refractory to infection, and that animals differ in the proportion of cells so protected by a uniform concentration of serum.

*The Weil-Felix Reaction in Proteus and Pyocyanus Infections.* By F. TREMAINE BILLINGS, JR. and GUSTAVE J. DAMMIN (introduced by Clifford L. Derick), Boston, Mass.

The present study was suggested by the observation that the sera of many patients with *Proteus* and *Pyocyanus* infections possessed high agglutinin titers for the Weil-Felix antigens. The incidence of positive Weil-Felix reactions was found to be higher in patients with these infections than in a large series of normal controls.

In a group of 9 patients, 8 with *Proteus* infections and 1 with a *Pyocyanus* infection, agglutinin tests were carried out with *Proteus* OX<sub>19</sub>, OX<sub>2</sub>, OXK and the infecting organism. Of this group, 5 possessed agglutinins for *Proteus* OX<sub>19</sub> in a serum dilution of 1:40 or higher, and also for the infecting organism in high titer; the remaining 4 failed to show agglutinins for either OX<sub>19</sub> or the infecting organism. Five patients agglutinated *Proteus* OX<sub>2</sub> in serum dilutions ranging from 1:40 to 1:5000, and 8 agglutinated *Proteus* OXK in serum dilutions of 1:80 to 1:2560. The sera of these 9 patients were absorbed with the infecting organism and in each instance it was possible to remove partially or completely the Weil-Felix agglutinins, indicating the presence of a common antigen.

Rabbits immunized with these 9 strains developed strong Weil-Felix reactions. Six agglutinated *Proteus* OXK in dilutions of 1:2560 or higher; 7 agglutinated *Proteus* OX<sub>2</sub> in serum dilutions ranging from 1:40 to 1:10000, and one rabbit developed a high agglutinin titer for *Proteus* OX<sub>19</sub>.

In each instance it was possible, with the immunizing organism, to absorb out the Weil-Felix agglutinins.

It is concluded that a positive Weil-Felix reaction may develop during the course of *Proteus* or *Pyocyanus* infections. In view of the general use of the Weil-Felix reaction in the diagnosis of rickettsial diseases, the results here reported suggest the need of greater care in interpreting this non-specific test.

*Solubility Studies on the Oral Administration of Sodium Sulfapyridine.* By SIDNEY S. SOBIN (introduced by Arthur C. Curtis), Ann Arbor, Mich.

Oral therapy with sodium sulfapyridine has been recommended by Ratish, Davidson, and Bullowa (J. Pharm. and Exper. Therap. 1940, 69, 365) as a procedure for rapidly achieving high blood levels. This procedure must assume an alkaline gastro-intestinal medium to maintain the sodium salts in solution. We have investigated the neutralizing effects of gastric juice on sodium sulfapyridine and the clinical absorption of these drugs.

Electrometric titration and quantitative sulfonamide determination of a 5 per cent solution of sodium sulfapyridine with gastric juice demonstrated that precipitation of the free base occurs with small amounts of gastric juice and is complete when the pH of the solution drops below 8.5 to 9.0.

Equivalent amounts of sulfapyridine and sodium sulfapyridine in aqueous solution or acacia suspension were administered to a group of patients. Blood levels of free drug were similar with both compounds. Some patients showed a more rapid rise but no higher peak (maximum level) with the sodium salt. Administration of equal amounts of sulfapyridine in tablet form and in finely powdered suspension again showed similar curves but more rapid absorption with the suspension. This suggests that particulate size may be an important factor in absorption.

Two patients showed an unexpected rapid high absorption curve with sodium sulfapyridine. Neither had free fasting HCl. Repetition of the sodium sulfapyridine administration, after histamine had produced acidity, showed a response similar to that of the free base. The pH of the gastric juice in this and other cases showed only slight elevation with oral sodium sulfapyridine.

*Discrepancies in the Inhibition-Concentration Relationship of the Sulfonamides.* By JEROME S. HARRIS and HENRY I. KOHN (introduced by David T. Smith), Durham, N. C.

In the case of certain sulfonamide drugs, e.g., sulfapyridine in pneumonia, it is becoming apparent that the therapeutic effect is independent of the dose or blood concentration over a fairly wide range. Although this paradox may be due to factors in the host or host-parasite relationship, it may be explained, in part, by the response of the bacteria to changes in drug concentration.

This may be demonstrated by comparing the inhibitions produced by varying concentrations of the sulfonamides on the growth rate of *E. coli* in proteose peptone medium.

Thus, raising the concentration of sulfapyridine from 2 to 60 mgm. per cent merely increased the inhibition of growth from 74 to 80 per cent. Above and below these levels, the inhibition increased rapidly with increase in drug concentration. Similar results were obtained with sulfathiazole and sulfadiazine. However, raising the concentration of sulfanilamide from 2 to 20 mgm. per cent increased the inhibition from 13 to 68 per cent. This suggests that raising the concentration of the sulfonamides, sulfanilamide excepted, may not produce the desired increase in therapeutic effect.

To explain these results, we suggest that the sulfonamides affect directly or indirectly a number of different bacterial metabolic systems at different concentrations. One such system, affected at low drug concentrations, is the synthesis of methionine in *E. coli*.

*Studies on Absorption of Sulfapyridine and Sulfathiazole.*

By J. MURRAY KINSMAN and (by invitation) JOHN WALKER MOORE, Louisville, Ky.

In 1939 we studied the absorption of sulfapyridine following the oral administration and following the rectal administration of its sodium salt. These studies were reported in part two years ago. In 1940, and to date in 1941, we have been studying the absorption of sulfathiazole. This has been administered orally and its sodium salt has been given rectally, intramuscularly and (lately) orally.

This report deals with a comparison of the absorption of the drugs under the above conditions.

Sulfapyridine is absorbed and excreted more slowly than sulfathiazole and maintains a higher blood concentration for equivalent dosage. Because of the rapidity of excretion of sulfathiazole, to be effective, doses have to be given at more frequent intervals than for sulfapyridine. By rectum, very high blood concentrations can be obtained and maintained with sodium sulfapyridine but not with sodium sulfathiazole. Intramuscularly, sodium sulfathiazole is slowly absorbed and gives blood concentrations much below what one would expect from corresponding oral doses of sulfathiazole; moreover, over a period of several days less than 25 per cent of the amount given can be recovered in the urine, indicating that some sort of protective coating may develop about the deposit in the muscle, preventing its ready absorption. The work on sodium sulfathiazole by mouth is in progress now, but so far it seems to indicate that the blood concentrations are lower than from the sulfathiazole itself.

Sulfathiazole was given to one patient following a total gastrectomy, and blood concentrations were below the expected level.

*Protective Activity of Normal Human and Animal Sera for Sulfapyridine-Treated Mice Infected with Pneumococci.* By SOPHIE SPICER (by invitation) and WHEELAN D. SUTLIFF, New York, N. Y.

Using mice fed a diet containing 1 per cent sulfapyridine as test animals, blood sera from other mice, guinea pigs, rabbits, rats, dogs, infant humans and adult humans

were examined to find the relative activity of these sera in protecting against pneumococcus infection, Type I.

The sera of dogs and adult humans increased the survival rate of sulfapyridine-treated mice. The sera of mice, guinea pigs, rabbits, rats and human infants did not increase the survival rate of sulfapyridine-treated mice.

Further studies of fresh serum as compared with heated or "old" serum showed that the protection partially disappeared on heating or on standing. The residual activity in "old" serum was destroyed by absorption with homologous type-specific pneumococcus cultures.

*Variations in Complement Activity of Blood Serum in Pneumonia.* By DAVID D. RUTSTEIN and WILLIAM H. WALKER (introduced by L. Whittington Gorham), Albany, N. Y.

The complement activity of 34 specimens of blood serum from 25 normal individuals and all but one of 47 specimens from 47 pneumonia patients following recovery fell within the narrow range of 0.00226 to 0.00660 ml.

The blood serum of 10 of 62 patients (16 per cent) showed, at the time of admission to the hospital during pneumonia, a marked drop ranging from 0.00773 ml. to complete failure of hemolysis with the largest amount of serum tested, i.e., 0.2 ml. of undiluted serum.

The serums from 7 of 12 pneumonia patients observed immediately before and after the administration of the initial therapeutic dose of antipneumococcal serum showed a striking drop, following serum administration, to a similar low range of complement activity.

The serums from all of 12 pneumonia patients observed immediately before and after the administration of the initial therapeutic dose of sodium sulfathiazole or sodium sulfadiazine showed no significant changes in complement activity following serum administration.

During serum sickness, following recovery from pneumonia, serums from 6 of 11 patients showed similar sharp decreases in complement activity which returned to normal following recovery from serum disease.

Except in one instance, the complement activity of guinea-pig serum was not inhibited by these serums of low titer after heat inactivation.

*Studies on Leptospiral Infections.* By THOMAS G. WARD (by invitation) and THOMAS B. TURNER, Baltimore, Md.

Since September of last year *Leptospira icterohemorrhagiae* has been recovered from three cases of Weil's disease in Baltimore. Two of these patients, brothers, aged nine and eleven, were infected by drinking water from a well in which leptospira virulent for guinea pigs were repeatedly demonstrated. Another case was traced to the same source. The third patient from whom the organism was recovered worked as a chicken picker in an establishment where rats were abundant. Virulent leptospira were demonstrated in the rats.

Agglutination and complement fixation tests have been made on sera from over 400 persons belonging to various groups in Baltimore with the following result:

Group tested	Number tested	Per cent positive
Chicken pickers .....	74	18.9
Meat packers .....	48	6.2
Candy makers .....	24	0
Medical students .....	66	0
Hospital patients .....	164	4.9
Miscellaneous .....	29	20.1
Total .....	404	7.7

The complement fixation is positive in only about one-half the sera showing positive agglutination. Rarely are complement fixing antibodies demonstrable in the absence of agglutinins.

Previous studies have shown that approximately one-third of the wild rats in Baltimore harbor *L. icterohemorrhagiae*. The foregoing data indicate that, in occupations entailing immersion of the hands in water open to contamination by rats, the risk of leptospiral infection is considerable.

Five strains of *L. icterohemorrhagiae* isolated locally from three human cases, a rat, and well water, respectively, show cross agglutination and complement fixation in high titer, and differ markedly in this respect from a strain of *L. canicola* isolated from a dog.

The following conclusions may be drawn:

1. Weil's disease is more common in Baltimore than previous records indicate.
2. The risk of infection is greatest among persons whose work brings them into contact with water contaminated by rat urine.
3. The agglutination reaction is commonly positive in higher titer, and over a longer period of time than is the complement fixation test.

*Sensory Neuron Degeneration in Young Pigs. Protection by Various Liver Extracts and Certain Vitamins. Antianemic Effectiveness of Livers of Ataxic and Non-ataxic Pigs.* By M. M. WINTROBE and (by invitation) CECIL MUSHATT, JOSEPH L. MILLER, JR., LAWRENCE C. KOLB, and H. J. STEIN, Baltimore, Md.

The observed efficacy of whole liver in protecting pigs against degenerative changes in the nervous system led to the investigation of the value of the various fractions of whole liver obtained during the manufacture of parenteral liver extract. The most effective fraction was an alcohol-soluble filtrate obtained after passage through permutit (parenteral anti-pernicious anemia liver extract, Parke Davis); the remaining fractions were relatively less effective in the following order: the residue after acid and heat treatment of whole liver ("press cake"), the fraction insoluble in 70 per cent alcohol ("Whipple" fraction) and the permutit absorbate. None of the fractions gave complete protection in all pigs in the doses used.

Preliminary observations suggest that certain of the newly synthesized "B<sub>2</sub> complex" vitamins afford protection. The content of these vitamins in the various liver fractions has been assayed.

In assays which are not yet complete the livers of ataxic pigs seem to be less effective in the treatment of pernicious anemia than those of normal pigs.

The relationship of these observations to pernicious anemia is discussed.

#### *The Chemical Determination of Nicotinic Acid Deficiency.*

By HENRY FIELD, JR., DANIEL MELNICK, W. D. ROBINSON, and C. F. WILKINSON, JR., Ann Arbor, Mich.

The application of the cyanogen bromide reaction which we have used yields values for blood nicotinic acid higher than those reported by other chemical methods and in good agreement with determinations by different biological methods. Confirming most of the reports of determinations by biological methods, we have not found any significant difference in the nicotinic acid contents of the bloods of normal and deficient subjects. Using various types of dosing for "saturation tests," we have not found that nicotinic acid is removed from the blood of pellagrins faster than from that of normal subjects.

The determination of nicotinamide in urine is complicated by the presence of other pyridine compounds which react similarly. Trigonelline, the methylated form in which the greater part of nicotinic acid is excreted, occurs preformed, without antipellagra activity, and is widely distributed in vegetable materials, predominantly coffee. Depending upon the individual, a variable fraction of nicotine is excreted which reacts like nicotinic acid. A larger fraction of nicotine excreted behaves on hydrolysis like trigonelline. The hydrolytic behavior of trigonelline permits its differentiation from other pyridine compounds and at least semi-quantitative analysis.

Twenty-four-hour urine specimens collected during abstinence from coffee, tobacco, tea and chocolate have shown a secretion of nicotinic acid averaging distinctly less in deficient than in normal subjects, but not consistently less.

The difference in the urinary excretion of trigonelline in normal and deficient subjects was much greater and much more consistent. It appears that, with a standardized basal regime and a more quantitative recovery, the urinary excretion of trigonelline may be the best chemical indication of nicotinamide deficiency.

#### *The Effect of Unilateral Paralysis of the Diaphragm on the Respiratory Mechanism.* By GEORGE W. WRIGHT and WARRINER WOODRUFF (introduced by William S. McCann), Trudeau, N. Y.

The effect of paralyzing one-half of the diaphragm on the rôle played by each lung in regard to minute ventilation, oxygen consumption, and carbon dioxide output has been studied previously in animals. Unsatisfactory technique, differences in the anatomy of the mediastinum and possible differences in the relative rôle played by the diaphragm in normal respiration have strongly militated against applying these observations to the human subject. Using the technique of bronchospirography as described by Jacobaeus and modified by Gebauer, we have studied the minute ventilation, oxygen consumption and carbon

dioxide output of each lung simultaneously with the patient at rest before and after paralysis of one-half of the diaphragm. In each of the five cases there was a reduction (12 to 21 per cent) in the minute ventilation, oxygen consumption and carbon dioxide output on the paralyzed side. No significant change was found in the arterial hemoglobin saturation. The vital capacity tended to be slightly reduced, although the maximum minute ventilation showed no reduction. The resting respiratory rate was increased moderately.

*Effect on the Lung Volume of Postural Changes in Patients with Orthopnea Due to Congestive Failure.* By M. D. ALTSCHULE and (by invitation) N. ZAMCHECK and A. IGLAUER, Boston, Mass.

A number of authors have attempted to explain the orthopnea of cardiac decompensation on the basis of increased congestion of the lungs when the patient assumes the recumbent position. Variations in the degree of pulmonary congestion should cause changes in the volume of the pulmonary air. Accordingly, the various subdivisions of the total lung volume were measured by the method of Christie and Meakins in various positions in patients with congestive failure and orthopnea. Only patients with mild or moderate orthopnea could be studied, since severe orthopnea made it impossible to secure satisfactory measurements.

In the patients studied the volume of the residual air was not changed in recumbency. It is clear that increased dyspnea in the recumbent position was not due to an increase in the degree of pulmonary congestion. Similarly, the partial relief of dyspnea obtained by assuming the upright position was not due to lessened pulmonary congestion. Other pulmonary factors are, however, important in the genesis of the orthopnea of congestive failure. Decreases in reserve air occur regularly when the recumbent position is assumed. This decrease in reserve air has been reproduced in the upright position by applying a tight abdominal binder and therefore appears to be the result of elevation of the diaphragm by the abdominal viscera. This elevation of the diaphragm impedes respiratory action and also makes the intrapleural pressure more positive, thereby further increasing the work that must be done to maintain a given level of respiratory activity. It is not to be inferred that orthopnea is due solely to these mechanisms; additional studies have stressed the rôle of intracerebral factors in its genesis.

*Mechanisms of Respiratory Failure under Barbiturate Anesthesia (Evipal, Pentothal).* By HENRY K. BEECHER and (by invitation) CARL A. MOYER,\* Boston, Mass.

Although the variability of effect of the barbiturates is well known, its causes are obscure. Since respiratory failure plays an important part in death under these agents, the respiration has been used as a means of investigating, in dogs, several factors responsible for the

variability of barbiturate effect. Evipal and pentothal have been studied. Since the barbiturates are *qualitatively* very similar in their actions, it is probable that the mechanisms described here apply to many members of the group. The respiratory response to the barbiturates when the blood oxygen is below normal, and the effect of low oxygen in temporarily masking serious overdosage, have been observed and considered in detail. The effect of a high oxygen content of the blood in depressing respiration under the barbiturates has been confirmed. The serious possibilities inherent in this action are now evident when considered together with the loss of sensitivity of the respiratory center to carbon dioxide under the barbiturates. This loss of sensitivity of the respiratory center results in a dangerous piling up of carbon dioxide in the blood. An increased sensitivity to small doses of barbiturates was observed when the blood carbon dioxide level was elevated. Finally, it was found that reflex respiratory failure could be produced by a slight increase in pressure in the airway when the subjects were deeply anesthetized and breathing one hundred per cent oxygen. This study provides information which helps to explain obscure accidents, particularly "sudden death without warning," under these agents and furnishes surer guides than have been available for the prevention of accidents in the future.

*Pathogenetic Mechanisms in Hemolytic Anemia.* By WILLIAM DAMESHEK and (by invitation) EDWARD B. MILLER, Boston, Mass.

Hemolytic anemia develops when certain blood-destroying factors overbalance those concerned with blood formation. Previous studies indicated (a) that hemolysins are important in both clinical and experimental hemolytic anemias and (b) that spherocytosis and increased hypotonic fragility are probably the result of hemolysin activity upon red cells *outside* the bone marrow.

"Hypersplenism" may explain some cases in which splenectomy results in cure and in complete disappearance of spherocytes. The stasis-agglutination theory of Ham and Castle fails to explain the presence of easily demonstrable hemolysins in various cases and the absence of hemolytic anemia in conditions of marked stasis, *i.e.*, polycythemia vera and splenic vein thrombosis.

Our recent experiments demonstrate that the normal erythrocyte may be damaged by (a) "simple" hemolysins which act directly (b) by "complex" hemolysins, including first "sensitization" of the red cell, then hemolysis due to complement activity and (c) by agglutinins. Red cells sensitized either by complex hemolysins or agglutinins are very fragile to mechanical means and thus liable to ready hemolysis in the circulation. Incompletely hemolyzed red cells—spherocytes—possess a diminished resistance to stasis. The factor of stasis appears to be less important than that of mechanical fragility or complement activity.

Hemolytic syndromes are due to a variety of agents—

\* Fellow of the National Research Council.



hemolysins, agglutinins, hereditary factors—which injure the red cell and make it vulnerable to erythrosthesis, mechanical trauma in the circulation, complement activity, etc. In testing red cells in hemolytic anemia, the factors of mechanical fragility, reaction to abnormal pH, etc. may be as important as that of an abnormal hypotonic fragility.

*Hemolytic Action of Certain Organic Oxidants Derived from Sulfanilamide, Phenylhydrazine and Hydroquinone.* By CHARLES P. EMERSON (by invitation), THOMAS HALE HAM and WILLIAM B. CASTLE, Boston, Mass.

In order to explain the mechanism of the increased osmotic fragility of red blood cells, hemoglobinemia and hemoglobinuria observed in the acute hemolytic anemia of certain patients receiving sulfanilamide, the effect of possible metabolic derivatives of sulfanilamide, including *p*-aminophenol and phenylhydroxylamine, was investigated *in vitro* and in cats and compared to the effect of phenylhydrazine, hydroquinone and some related compounds.

Human or cat erythrocytes were incubated for from 4 to 24 hours at 37° in serum or in buffered salt solution (pH 7.4) in the presence of oxygen with and without each drug in a concentration of 4.5 millimoles. Those compounds, or their derivatives, which may form oxidants in oxidation-reduction systems, namely, hydroquinone, *p*-aminophenol, hydroxylamine, phenylhydroxylamine and phenylhydrazine, caused the formation of methemoglobin, an increase in volume and osmotic fragility of the red cells eventually resulting in hemolysis. The effects of *p*-aminophenol and hydroquinone on osmotic fragility were completely inhibited by anaerobic conditions and that of hydroquinone was decreased in proportion to the tension of carbon dioxide. Certain organic compounds of related structure, which presumably do not act as oxidants under the conditions employed, failed to produce increased fragility of the erythrocytes when used in equimolar concentrations. These included sulfanilamide, sulfanilic acid, *p*-nitroaniline, *p*-aminobenzoic acid and phenol.

Each of the above compounds causing changes *in vitro*, when injected intraperitoneally into cats in doses of from 6 to 50 mgm. daily, produced changes in the peripheral blood which paralleled those found in certain cases of acute hemolytic anemia due to sulfanilamide, including the formation of methemoglobin, rapid development of anemia with hemoglobinemia and so great an increase in fragility that erythrocytes were hemolyzed *in vitro* in salt solutions approaching isotonicity. Sulfanilamide and *p*-aminobenzoic acid, when injected in similar doses, produced only moderate anemia and no significant change in erythrocyte fragility.

It is inferred that the hemolytic activity of compounds such as sulfanilamide, hydroquinone, *p*-aminophenol, phenylhydroxylamine and phenylhydrazine is due to the formation *in vivo* of certain oxidative derivatives which *in vitro* were demonstrated to cause increased erythrocyte fragility.

*Leukopenia. The Rate of Disappearance of White Blood Cells from the Peripheral Blood in Leukopenic States.* By JOHN S. LAWRENCE and (by invitation) DONALD M. ERVIN and RAYMOND M. WETRICH, Rochester, N. Y.

Cats which have been leukopenic due either to infectious feline agranulocytosis or irradiation of the bone marrow have been connected by means of an end-to-end anastomosis of the carotid arteries with normal animals. When thorough mixing of the blood has occurred, the animals have been disconnected and allowed to go on their own circulation. Following this, the normal animals have promptly developed a marked leukocytosis with a distinct "shift to the left," whereas the leukopenic animals have shown a rapid diminution in the total number of white blood cells in the vascular channels until their original leukopenic levels have been reached. The white blood cells disappear at the rate of approximately 1000 cells per cu. mm. per hour. Examination of sections from various tissues of these animals has failed to show any accumulation of white blood cells that would explain their disappearance from the blood.

As a working hypothesis, we have suggested that white cells normally stay in the blood only a short time, the blood serving merely as a means of transporting them to the various tissues of the body. Whether there is such a rapid disappearance of cells from the blood channels of normal animals cannot be said, but it is certainly true that in leukopenic animals of the types we have used there is a rapid disappearance of white blood cells from the vascular channels.

*The Management of Hemophilia with Lyophilized Human Plasma Intravenously.* By JOHN B. JOHNSON (introduced by Samuel H. Bassett), Rochester, N. Y.

Five hemophilic patients have been studied. Evidence has been obtained to show that lyophilized plasma causes a similar reduction in the coagulation time to that caused by the transfusion of whole blood. The potency of the dried plasma has been shown to be greatest when it is prepared immediately after the blood is drawn from the donor. The sources of plasma have been patients with polycythemia vera or with acute left heart failure, and friends of hemophilic patients. No typing is necessary.

Using dried plasma only, tooth extractions have been made in two patients. Massive hematuria in one patient was practically cleared 48 hours after two doses of plasma, and 12 hours later the guaiac was continuously negative.

One of our patients had led a wheel chair existence for 3 years because of frequent recurrence of hemarthroses when walking. With weekly injections of plasma and proper muscular exercises, he began walking. For two months on this treatment no hemorrhages occurred, and the patient secured a steady job. Plasma was then discontinued for one month, during which time one traumatic and two spontaneous hemorrhages occurred (one a massive hematuria).

Dried plasma offers a simplified method for managing the emergencies of hemophilia and also a relatively in-



expensive replacement therapy by which the hemophilic patient may be rehabilitated into the community.

*The Detection of Abnormal Erythrocytes by Opacity Measurements of Blood in Varying Concentrations of Saline.* By BENJAMIN ALEXANDER (introduced by Samuel L. Gargill), Boston, Mass.

The opacity of saline solutions of normal red cells and cells in pathologic conditions has been measured with the Evelyn photoelectric colorimeter.

When a given number of normal red cells is suspended in 2.5 per cent saline solution the opacity is  $83 \pm 3$  per cent greater than when the same number of red cells is suspended in physiological saline. In normal blood, therefore, the increase in opacity in the 2.5 per cent saline suspension is proportional to the number of red cells suspended. In certain pathological conditions, on the other hand, the red cell opacity in the two media did not show the same change as in normal blood suspension of the same number of red cells. It was found that 84 per cent of those cases showing a red cell opacity change greater than normal in suspensions of a given number of red cells had clinical conditions known to give rise to macrocytic anemia. In many instances, abnormally large opacity change was associated with a demonstrable increase in the mean corpuscular size. In some instances in which the opacity change was abnormally great, however, no abnormality of the blood could be demonstrated. In certain of these cases the opacity change became normal after liver therapy.

It would appear that this measurement of the opacity difference of a given number of red cells in physiological saline and in 2.5 per cent saline solution can be utilized to reveal a fundamental abnormality of the red cells. Thus far, most of the cases in which this abnormality has been detected have had clinical conditions known to be associated with macrocytosis.

*An Analysis and Correlation of Hemocytologic with Other Significant Reactions in Monkeys Following Simultaneous or Successive Inoculations with the Virus of Epidemic Influenza and Hemolytic Streptococci.* By CHARLES A. DOAN and (by invitation) ORAM C. WOOLPERT, CESAR MERINO, J. L. SCHWAB, and S. SASLAW, Columbus, O.

The virus of epidemic influenza (PR8 strain of Type A) and the streptococcus hemolyticus (Group C) were administered either simultaneously or successively, by the nasal route, to monkeys (*Macacus mulatta*) under ether anesthesia. The clinical, hemocytologic, serologic, and other pertinent reactions were followed until recovery (or rarely death) occurred. The order of administration of the two agents was reversed in separate series of animals and the interval between inoculations was varied. In selected instances, recovered animals which had received one or both infecting agents were later reinoculated and comparative observations were recorded.

Primary inoculation with the streptococcus was characterized by an immediate polymorphonuclear leukocy-

tosis, with little or no change in the opsonocytophagocytic index. Primary inoculation with the influenza virus, on the other hand, was followed promptly by a granulopenic leukopenia, with specific virus-neutralizing antibodies demonstrable a little later. When the virus was given concurrently with the streptococcus, or shortly thereafter, the streptococcus-stimulated granulocytosis developed first, followed after several days by a latent virus-induced leukopenia. If inoculation of the streptococcus following the virus infection was delayed, no leukocytosis developed.

On reinoculation with streptococcus several months after the primary inoculation, no significant leukocytosis developed but a marked increase in the opsonocytophagocytic index was noted very promptly. Readministration of virus to these animals seemed to suppress this index. Marked anemia of the hypochromic, microcytic type developed in the reinoculated animals, even though the plasma iron remained high. Hypertension, edema, albuminuria, red cells and urinary casts were observed in a number of monkeys reinoculated with hemolytic streptococci. The streptococci tended to persist or recur in the throats of animals thus affected.

*The Thrombic Activity of a Fraction of Rabbit Plasma Globulin.* By EUGENE L. LOZNER and F. H. L. TAYLOR (by invitation) and MAXWELL FINLAND, Boston, Mass.

A globulin fraction of rabbit plasma isolated by the technique of Parfentjev was shown to have a marked effect on reducing the coagulation time of both normal and hemophilic blood *in vitro*. This substance clotted both citrated and oxalated blood. The conversion of fibrinogen into fibrin by this substance was independent of calcium and prothrombin concentrations. This substance therefore has thrombic activity. Preliminary observations on the control of bleeding in normal and hemophilic subjects indicate that this globulin fraction has considerable hemostatic activity.

*Study of the Coagulating Action of Fer-de-Lance Venom.*

By GEORGE L. KAUFER, JR. and ROBERT M. BIRD (by invitation) and PAUL REZNIKOFF, New York, N. Y.

A study was made of the blood-coagulating action of a fresh saline solution of crystalline "detoxified" fer-de-lance venom. *In vitro*, oxalated and heparinized human and dog blood was used; *in vivo*, dogs and rabbits were used.

*In vitro* observations revealed a more rapid clotting action on oxalated than on heparinized blood. Using "pure" fibrinogen solution, rapid conversion to fibrin occurred. Lysis of the fibrin occurred in the presence of high concentrations of venom, but this was not appreciable in lower concentrations.

Intramuscular and intravenous injections of varying venom concentrations in rabbits resulted in prolonged clotting time and reduction of fibrinogen blood levels. The intravenous injection of 0.1 cc. 1 per cent venom in dogs caused the clotting time to become indefinitely prolonged with complete disappearance of prothrombin and

of fibrinogen. In animals surviving, the clotting time, prothrombin and fibrinogen slowly returned to normal. At no time was a "positive phase" of coagulation found.

An anti-venom rabbit serum was produced by repeated subcutaneous injections of venom. This serum protected dogs from lethal doses of venom, and even from the anticoagulant action of sublethal doses. *In vitro*, venom mixed with this rabbit serum was unable to clot oxalated blood.

*Hemoglobin Regeneration in Blood Donors.* By W. M. FOWLER and (by invitation) A. P. BARER, Iowa City, Ia.

Observations have been made on the rapidity of hemoglobin regeneration in 200 blood donors who have given blood for a total of 636 transfusions. The withdrawal of 500 to 600 cc. of blood caused an average drop of 2.325 grams in the blood hemoglobin. The average time necessary for the hemoglobin to return to its original level was 49.6 days with a range of 18 to 98 days. There was a definite linear relationship between the drop in hemoglobin and the recovery period in days in spite of the great individual variations. At the end of 8 weeks 74.2 per cent of the donors had regained their original hemoglobin level but the remaining 25.8 per cent required up to 15 weeks for complete recovery.

The withdrawal of 300 cc. of blood caused an average fall of 1.238 grams in the blood hemoglobin and the average recovery period was 33.3 days.

The average rate of hemoglobin production was 0.0495 gram per day in males but was somewhat slower in females, 0.040 gram per day.

Hemoglobin regeneration was no slower after subsequent donations than it had been after the first.

From these data it is apparent that a 3-month interval is advisable between blood donations unless it is established that the blood hemoglobin has returned to its original level before this time. Subsequent donations after complete recovery did not lead to a diminished rate of hemoglobin regeneration.

*Leukocytosis in Patients with Psychiatric Disorders.* By S. M. SMALL and O. DIETHELM (by invitation) and A. T. MILHORAT, New York, N. Y.

Leukocytosis is of frequent occurrence in patients with emotional disorders. Most of these patients show no infectious process or physical disease to account for the elevated white cell count. Briefly, the observations on 200 psychiatric patients were as follows: There was no definite correlation between the level of the white cell count and specific psychiatric disease entities. On the other hand, in certain patients with elevated white cell counts the degree of leukocytosis was often related to the intensity of the psychopathologic emotion. This relationship was consistent in the same subject but varied from patient to patient. The specific symptoms most frequently associated with leukocytosis were panic reactions, agitation, excitement with overactivity, and anxiety. Improvement in the emotional reactions, either spontaneous or induced by sedation, was associated with a return of the

leukocyte count to normal levels. The intravenous administration of sodium amytal induced striking reductions in elevated leukocyte counts when there were accompanying favorable changes in the emotional status. The specific gravity of the blood plasma often varied in patients with leukocytosis, and usually was reduced by intravenous sodium amytal; however, changes in white cell counts and plasma specific gravity occurred independently of each other.

*The Effect of Alterations of the Acid-base Balance Upon CO<sub>2</sub> Output by the Lungs.* By JACK D. ROSENBAUM (by invitation) and JOHN P. PETERS, New Haven, Conn.

Respiratory CO<sub>2</sub> output with changing acid-base balance was studied in human subjects. During overventilation, a small CO<sub>2</sub> loss from the extracellular fluids (ECF) is associated with a large increase in expired CO<sub>2</sub>; whereas ammonium chloride acidosis causes considerable decrease in ECF CO<sub>2</sub> content with little change in CO<sub>2</sub> ventilation. This suggests that tissue CO<sub>2</sub> content may change independently of alterations in total CO<sub>2</sub> concentration of the ECF.

This hypothesis is confirmed by observations that intravenously administered bicarbonate is distributed through the ECF alone, indicating impermeability of tissue cells to HCO<sub>3</sub><sup>-</sup> ion. Molecular CO<sub>2</sub>, however, has been shown by others to diffuse through total body water. Consequently, tissue CO<sub>2</sub> content varies with CO<sub>2</sub> tension of the ECF, but not with HCO<sub>3</sub><sup>-</sup> ion concentration.

During overventilation, although serum total CO<sub>2</sub> content falls but slightly, serum CO<sub>2</sub> tension decreases markedly; large amounts of CO<sub>2</sub> enter the blood from the tissues to be lost through the lungs. Conversely, if serum CO<sub>2</sub> tension rises following NH<sub>4</sub>Cl ingestion, CO<sub>2</sub> diffuses from blood to tissues and does not appear in the expired air.

Evaluation of the influence upon respiratory exchange of acid-base alterations necessitates analysis of the mechanisms of each change.

*Vitamin A Metabolism in Obstructive Jaundice.* By JOHN D. STEWART and G. MARGARET ROURKE (introduced by Allan M. Butler), Boston, Mass.

The relationship between concentration of vitamin A and carotenoids in the plasma and vitamin A in the liver has been studied. Biopsy of liver tissue has been done at operation on patients with liver disease (obstructive jaundice) and on control patients without liver disease. The patients with obstructive jaundice frequently showed zero values in plasma and liver. By giving massive doses of vitamin A parenterally before operation the hepatic content could be brought to normal, but the plasma concentration rose more slowly. The presence of normal plasma carotenoid concentration and reduced vitamin A concentration probably indicated liver damage and failure in conversion of the provitamin. Disturbances in absorption and conversion of carotene, and in storage of vitamin A, may occur in obstructive jaundice. No close correlation was found between plasma and hepatic concentrations.

*The Collection and Analysis of Fluid from Single Nephrons of the Mammalian Kidney.* By ARTHUR M. WALKER and (by invitation) PHYLLIS A. BOTT, JEAN OLIVER and MURIEL C. MACDOWELL, Philadelphia, Pa., and Brooklyn, N. Y.

A technique has been developed for visualizing the surface of the mammalian kidney and for collecting fluid from single glomeruli, and proximal and distal convoluted tubules which appear there. Sufficient fluid (0.1 to 1.0 c.mm.) can be collected to permit quantitative analysis by methods which proved serviceable in a similar type of experimentation on the amphibian kidney. The site of the fluid collection is determined by macerating the kidney and isolating the punctured nephron in its entirety by microdissection.

Sixty successful experiments have been performed on rats, guinea pigs, and opossums. Glomerular fluid proves to be free from detectable amounts of protein, to have the same vapor pressure as blood plasma, and to contain glucose and exogenous creatinine in the same concentrations that exist in plasma water. The proximal tubules not only reabsorb glucose in these animals, as they do in amphibia, but also reabsorb about 80 per cent of the fluid in glomerular filtrate. This fluid reabsorption is accomplished without any increase in vapor pressure of the tubule contents but it is not a reabsorption of undifferentiated glomerular fluid, for the chloride concentration of proximal tubule fluid is half again as high as that of blood plasma. The results of the chloride analyses imply the preferential reabsorption of some other anion ( $\text{—HCO}_3^-$ ) by the proximal tubule.

*Acidosis in the Premature Infant.* By WILLIAM STERRY BRANNING (introduced by WILBURT C. DAVISON), Durham, N. C.

Acid-base determinations done on fifteen apparently well infants whose birth weight was below 2250 grams showed that, on a diet of 120 calories per kilo per day, plasma bicarbonate was consistently lower in them than in infants of normal birth weight, and the organic acids in blood amounted to approximately three times the normal value. Total base, chloride, and phosphate all remained within the normal range.

High organic acid values in blood are supported by the fact that organic acid excretion in urine averaged 2.6 m. eq. per kilo per day as opposed to the "normal" range of 0.6 to 1.0 m. eq. per kilo per day for adults. All urine specimens taken had low pH values, averaging pH 5.27. The titrated total organic acids averaged 55.6 mm. eq. per liter; of this, lactic acid composed 10 to 20 per cent, uric acid 2 to 4 per cent.

Determinations made on six infants with clinical symptoms of acidosis showed total base, chloride, and phosphate all within the normal range. Bicarbonate ranged from 9.0 to 16.0 m. eq. per liter plasma; calculated organic acids 11.6 to 28.0 m. eq. per liter. Organic acid excretion varied from 22 to 100 m. eq. per liter.

*Conclusions.* The organic acid constituents of plasma and urine of premature infants are usually two to three

times that considered normal, and plasma bicarbonates are correspondingly low. In blood and urine, lactic acid and uric acid account for, at most, 15 to 25 per cent of the organic acid present. The premature infant is always on the borderline of acidosis.

*Studies on Experimental Hypochloremia in Man.* By JOSEPH B. KIRSNER and KATHRYN KNOWLTON (by invitation) and WALTER LINCOLN PALMER, Chicago, Ill.

In connection with certain studies on alkalosis previously reported, one of us (J. B. K.) produced severe hypochloremia and alkalosis without marked hyperazotemia in the dog by the daily withdrawal of gastric juice. No definite functional or anatomic evidence of renal injury was noted in these animals. The present report deals with a hypochloremia (ca. 60 mM./L.) similarly produced in man. A state of acute collapse resembling in some respects an Addisonian crisis is described. Detailed studies of the blood electrolytes disclosed such abnormal values as:

Serum Cl 54–60 mM./L. (normal 99–108)  
 Serum  $\text{CO}_2$  40–44 mM./L. (normal 22–30)  
 Serum pH 7.60–7.70 (normal 7.35–7.48)  
 Na 115–130 mEq./L. (normal 137–147)  
 Total base 134–146 mEq./L. (normal 150–160)

The blood urea nitrogen and the urea clearance were not altered significantly. The volume of urine excreted was large; at times the urine contained no chloride and almost no sodium. Dehydration, as evidenced by a high red blood cell count, increased hematocrit readings, plasma protein values, and a decrease in body weight, was present despite the administration of adequate amounts of fluid. This was due apparently to the inability of the body to retain water.

The chloride content of the gastric secretion was not altered appreciably.

These studies further emphasize the importance of the electrolytes of the blood in the maintenance of normal water balance and also show that the profound alterations seen in alkalosis secondary to chloride loss are independent of demonstrable renal injury.

*Studies on the Excretion of Bromsulfalein in the Bile.* By A. CANTAROW (introduced by Hobart A. REIMANN), Philadelphia, Pa.

After intravenous injection of bromsulfalein (2 mgm. per kgm.), the curve of its excretion in liver bile was determined by the use of the Evelyn photoelectric colorimeter. Studies were performed on bile-fistula dogs and human subjects. Normally, the dye appeared in the bile within 15 minutes and reached a maximum concentration in 45 to 60 minutes, falling to a low level at 2 hours, and often not disappearing completely for 5 to 6 hours. Normally, 35 to 83 per cent of the quantity injected was excreted in the first hour and 61 to 100 per cent in the first 2 hours. Abnormal excretion of the dye was evidenced by one or more of the following phenomena: (1) delayed removal from the blood; (2) delayed entrance

into the bile; (3) delayed attainment of maximum concentration; (4) prolonged high curve of excretion; (5) subnormal concentration; (6) abnormally low excretion within 1- or 2-hour periods after injection. Since 85 to 95 per cent of the dye leaves the blood within 5 minutes, this method permits practical study of two phases of bromsulfalein excretion: (1) the rate of its removal from the blood (reticulo-endothelial cells?) and (2) the rate of its passage into the bile (hepatic cells).

*Further Observations on the Oral Administration of Citrated Blood in Man.* By LEON SCHIFF and (by invitation) R. J. STEVENS, N. SHAPIRO, and S. GOODMAN, Cincinnati, O.

Studies were carried out in a group of thirty subjects who were given varying amounts of citrated human blood (from 1 cc. to 2000 cc.) by mouth or by stomach tube. Observations were made on the following points:

1. The amount of blood necessary to produce a positive chemical test for occult blood.
2. The amount of blood necessary to produce a tarry stool.
3. The number of tarry or bloody stools, and the duration of a positive chemical test for occult blood following administration of large quantities of blood.
4. Time required for blood in the stomach to assume "coffee-grounds" appearance.
5. Relationship to nausea and vomiting.
6. Effect on temperature, white and red cell counts.
7. Effect on icteric index and urobilinogenuria.

The clinical implications of these observations are discussed.

*Further Studies Concerning the Effects of Sulfanilamide on Acid-Base Metabolism.* By WILLIAM W. BECKMAN and OTTO KRAYER (by invitation) and WALTER BAUER, Boston, Mass.

Measurements of sodium and chloride excretion by the heart-lung-kidney preparation were made before and after the administration of sufficient sulfanilamide to produce a blood level of 20 mgm. per cent. Notwithstanding the very diminished excretion of sodium which these preparations regularly exhibit, following sulfanilamide there was a definite acceleration in the excretion of this ion. This increase was not accompanied by a similar increase in chloride excretion. Simultaneously in the serum there was a small drop in the concentration of sodium and bicarbonate and a rise in the concentration of chloride. The direction of these changes was the same as that previously described in human subjects receiving sulfanilamide. Hence these experiments define to some degree the search for the manner of production of the unusual type of acidosis encountered when sulfanilamide is given.

*Reactions of Patients with Malignant Tumors to Injection of Bacterial Filtrate.* By AUSTIN M. BRUES, Boston, Mass., and (by invitation), M. J. SHEAR, Bethesda, Md.

Four patients with inoperable malignancy were given injections of that fraction of B. coli filtrate which

pared by Shear and Turner, which produces hemorrhage in and, occasionally, regressions of animal tumors, particularly sarcoma (cf. Schwartzman reaction). All patients had a chill, transitory fever and leukocytosis. In one patient (multiple myeloma) the course of the disease appeared to be unaltered. In two patients (Ewing's sarcoma and metastatic carcinoma of prostate) there was immediate pain in tumor areas, followed by relief of symptoms for a time. One patient with lymphosarcoma, treated when in *extremis*, showed clinical evidence suggesting widespread destruction of tumors and remained well for some time. All patients died with malignancy. Two showed evidence of hemorrhage into tumors at post-mortem, although the causal relation of this to filtrate injection is not certain. Two patients showing considerable relief of symptoms rapidly developed high non-protein nitrogen (90 and 110 mgm. per cent) and uric acid (16 and 27 mgm. per cent) levels in the blood. One patient excreted, on a low purine diet, 2500 mgm. of uric acid in the first 24 hours. In two cases there was evidence of congestive failure following treatment. Successive treatments had little additional effect, even with increasing dosage. Response to waste products may be a major medical problem with any chemotherapeutic agent causing rapid destruction of tumor tissue.

*The Diagnostic Value of Synovial Fluid Examination.*

By MARIAN W. ROPES and HOWARD C. COGGESHALL (by invitation) and WALTER BAUER, Boston, Mass.

Detailed examinations of 750 synovial fluids from various types of joint disease have been made. Analyses of these data indicate that the majority of the fluids can be separated into two groups. Group I fluids are essentially traumatic in nature (traumatic arthritis, degenerative joint disease, Charcot joints and osteochondromatosis). Group II includes those obtained from specific infectious arthritides of known origin and rheumatoid arthritis.

Most Group I fluids are clear and do not clot, whereas Group II fluids may be clear or turbid and usually do clot. Total leukocyte counts above 1000 are rarely encountered in Group I fluids, whereas in Group II they are generally 3000 or higher. The polymorphonuclears are less than 500 in Group I and ordinarily greater than 1000 in Group II. The protein concentration is less than 5.2 grams per cent in Group I but varies from 3.0 to 8.9 grams in Group II. The changes in the globulin fraction are most significant. The mucin concentration is usually lower in Group II, but more important than the concentration are its precipitability with acetic acid and the viscosity of the fluid. The mucin precipitate from Group I fluids is ropey and the solution clear, while the precipitate from Group II fluids is usually friable and the solution cloudy. Group I fluids usually show only a moderate reduction of viscosity, as compared to a marked reduction in most Group II fluids. In Group I the serum-fluid fasting sugar difference is usually normal (less than 10 mgm. per cent) in contrast to a much greater difference in the majority of

To summarize, aspiration of a turbid fluid which clots and has a low viscosity, a total leukocyte count above 3000 with absolute polymorphonuclear count above 500, a protein content above 5.0 grams per 100 cc. and a serum-fluid sugar difference of over 20 mgm. per 100 cc. indicates that the joint disease is not in Group I.

Results in individual cases have shown that examination of joint fluid used in conjunction with history, physical examination and other laboratory tests is of definite diagnostic and prognostic value.

*Cytologic Study of Synovial Tissue in Various Types of Arthritis.* By CURRIER McEWEN and (by invitation) ERNST W. BERGMANN and HARRY MOST, New York, N. Y.

Synovial tissues obtained at operation have been studied by the supravital technique as well as with ordinary histologic methods. In 2 cases of relatively normal synovial tissue obtained at operation to correct traumatic disabilities, the cells found were relatively few in number and consisted chiefly of small fixed connective tissue cells. In 6 cases of osteoarthritis and traumatic arthritis of long duration in which synovial thickening was present, the cells were more numerous and were chiefly made up of fixed connective tissue cells of small to moderate size. In 6 cases of rheumatoid arthritis the cells were greatly increased in numbers and, while predominantly of moderately large fixed connective tissue type, included also mono- and multinuclear giant cells of the type previously shown to be present in the subcutaneous nodules of rheumatic fever and rheumatoid arthritis. In 13 cases of tuberculous arthritis the cells were very numerous and, while the majority of cells were of the moderately large connective tissue type found in the rheumatoid biopsies, none of the "rheumatoid giant cells" was found, but, instead, typical epithelioid cells as described by Sabin were noted.

*The Distribution and Rate of Formation of Synthetized Cholesterol.* By J. ROSCOE MILLER and ARNOLD WAGNER (introduced by N. C. GILBERT), Chicago, Ill.

These experiments were based on the fact that deuterium cannot be incorporated into the cholesterol molecule by *in vitro* or *in vivo* procedures, but that it can be incorporated into the cholesterol molecule during the formation of that substance in the body. This offers a means of identifying the rate and distribution of cholesterol synthetized subsequent to the introduction of heavy water into the body fluids. Rats were used as experimental animals and the mass spectrograph was used for the deuterium determinations. Individual animals were sacrificed at given intervals following the introduction of heavy water and the amount of tagged cholesterol in the individual organs was determined.

*Gold Metabolism and Excretion Studies in Patients Treated with Different Gold Salts.* By R. H. FREYBERG, and (by invitation) C. J. SMYTH and W. D. BLOCK, Ann Arbor, Mich.

Patients with rheumatoid arthritis were studied during

and for several months following the administration of gold sodium thiomalate (myochrysin), gold sodium thiosulfate and colloidal gold sulfide. The content of gold in the blood, urine and feces was measured. The effects of different gold salts were compared.

In general, with the use of gold sodium thiomalate and gold sodium thiosulfate, the blood concentration and excretion of gold were similar. The blood content and excretion of gold increased as the weekly dose of gold increased but not in direct proportion. Results obtained with colloidal gold sulfide were exceedingly variable but with few exceptions the blood and excretion values were much smaller than those obtained with other preparations. In every instance gold was retained in the body for many months after its administration ceased; the amount retained and the length of time varied in proportion to the weekly dose of gold.

Pertinent data obtained in animal investigations of deposition and toxicity of gold are correlated.

Results are discussed in relation to the therapeutic value and the toxicity of the various gold preparations and different administration techniques, and indications will be pointed out concerning the improved use of gold salts in treatment of arthritis.

*The Determination of the Relative Cerebral Metabolism in Man.* By EUGENE B. FERRIS, JR. and (by invitation) HENRY W. RYDER and NATHANIEL BROWER, Cincinnati, O.

A simple method for determining an index of the relative rate of intracranial blood flow in man has been developed. It consists in relating the rise of internal jugular venous pressure to pressure increments which are applied to the neck by means of a freely distensible cuff. The validity of the method has been tested by theoretical considerations, by checking it empirically against factors known to increase and decrease cerebral blood flow, and by comparing it with the blood flow as determined objectively by the cerebrospinal fluid displacement method. The results are reproducible in the same subject and reasonably comparable in different control subjects.

Analysis of internal jugular venous blood and of femoral arterial blood gives the arteriovenous oxygen difference. This, considered together with the "blood flow index," as obtained above, gives the relative rate of total cerebral aerobic metabolism.

In control subjects, the value for the rate of cerebral metabolism is more constant than either the blood flow or A-V difference. Illustrative examples of clinical states in which the relative total cerebral aerobic metabolism is not greatly altered and in which it is greatly reduced are presented.

There are no apparent contraindications to the procedure other than those against venous and arterial puncture and against elevating the intracranial venous pressure.

*The Clinical Significance of "Wandering" Auricular Pacemaker.* By ALEXANDER M. BURGESS, JR. (by invitation) and LAURENCE B. ELLIS, Boston, Mass.

A study of a series of 130 patients showing so-called "shifting" or "wandering" auricular pacemaker is reported. This investigation was undertaken in order to determine the clinical significance of this electrocardiographic phenomenon which, though not uncommon, has been neglected in the literature. The cases were found to fall into two types: a "simple" type, with a cyclic shift between two distinct auricular pacemakers, and a totally irregular type with numerous foci of impulse production giving rise to contractions in no regular sequence. There were 72 cases of the "simple" type, which were found to be distributed at random among patients with and without heart disease. All 52 cases of the totally irregular type were found to have significant cardiac abnormalities, and in 90 per cent of these cases the underlying pathology was coronary artery disease. Thus it is apparent that the simple type is not necessarily of pathological significance, whereas the irregular type is an indication of heart disease. The possible mechanism of production of this phenomenon is discussed.

*Observations on the Normal Mechanism of Closure of the Ductus Arteriosus.* By J. ALLEN KENNEDY and SAM L. CLARK (introduced by Hugh Morgan), Nashville, Tenn.

The normal mechanism of the closure of the ductus arteriosus is not known. Our work has been concerned with an attempt to elucidate this problem by observations on the ductus arteriosus of the guinea pig fetus. Both fixed and living preparations were studied.

Normal closure of the ductus arteriosus at birth involves two phases. The first is immediate and consists of a muscular contraction requiring several minutes which obliterates the ductus lumen. The ductus when once closed (phase one) remains closed and this occurs within about 5 minutes after birth in normal animals. A second slower phase requiring several weeks involves the transformation of the obliterated muscular ductus into the fibrous ligamentum arteriosum.

Physiological closure of the ductus arteriosus can be studied in guinea pig fetuses near term. The fetuses are delivered by hysterotomy beneath a bath of warm saline with the placental vessels intact. The ductus is visualized through an opening in the chest.

The ductus will close promptly following certain stimuli. Mechanical stimuli to the ductus, such as gentle pinching or tugging, and electrical stimulation of the ductus are followed by closure in 15 to 30 seconds. Artificial inflation of the lungs with air, injection of adrenalin, massage of the carotid sinus, and hemorrhage are followed by closure of the ductus in several minutes. Under proper conditions, withdrawal of the stimulus is followed by opening of the ductus. This process of closure and opening resulting from the application and withdrawal of certain stimuli can be repeated many times.

In view of these experiments it is suggested that a

patent ductus arteriosus may be due to the failure of a normal physiological mechanism rather than to a true developmental anomaly.

*Circulatory Failure in Acute Infections.* By RICHARD V. EBERT (by invitation) and EUGENE A. STEAD, JR. (introduced by Marshall N. Fulton), Boston, Mass.

The clinical course and hemodynamics of the circulatory failure produced by acute infections have been studied in eight patients. There were six cases of pneumococcus pneumonia, one each of streptococcus and staphylococcus septicemia. Clinically, these patients showed cold extremities, sweating, pallor, narrowing of the field of consciousness, small or absent pulsations of the radial artery, low arterial pressure, narrow pulse pressure, and tachycardia. The values for the plasma volume, the hematocrit, and the serum protein concentration, were within normal limits. The venous pressure was within normal limits before transfusion. In two patients auricular fibrillation developed after the onset of circulatory failure. Electrocardiograms failed to show any other significant variations from the normal.

The blood volume was increased by transfusions of whole blood or by administration of human plasma. Venous pressure readings were taken during the administration of the fluid, and in four cases sufficient fluid was given to increase the venous pressure. Hematocrit and serum protein determinations indicated a sustained increase in plasma volume. The arterial pressure in two cases showed a transient rise of 5 to 10 mm. of mercury, and the radial pulse was usually palpable for a short time. There was no other evidence of clinical improvement and, although the plasma volume remained increased, the arterial pressure returned to its former level.

Although, clinically, these cases with acute infection and circulatory failure resemble cases of traumatic shock, the data indicate that the pathogenesis is different from traumatic shock, because the patients with acute infections do not show a significant degree of hemoconcentration and they are not benefited by transfusions. The fact that no improvement occurred with elevation of the lower part of the body, and the fact that little improvement occurred even when the venous return to the heart was adequate, as demonstrated by a rise in venous pressure to abnormal levels following transfusion, indicate that peripheral pooling of blood is not the primary factor in causing the circulatory failure.

The failure of the circulation to improve when the venous pressure is abnormally increased by intravenous fluids is evidence that cardiac function is not normal. The fact that the venous pressure is not elevated before transfusion, even though the cardiac function is poor, is evidence that the venous bed is not responding with normal tone to a decrease in cardiac output. The experimental evidence suggests that the circulatory failure is produced by a combination of cardiac and peripheral factors.

*The Impaired Renal Excretion of Salt in Chronic Congestive Heart Failure.* By PALMER H. FUTCHER and HENRY A. SCHROEDER, New York, N. Y.

Passage of sodium chloride into the tissues in edema fluid has been regarded by many investigators as the explanation for retention of salt in chronic heart failure. The following experiments, however, suggest impairment of the ability of the kidney to excrete salt even when the blood salt level is artificially elevated above normal.

Patients maintained on a low salt diet were given intravenously 20 to 30 grams of sodium chloride as a 6 per cent solution. Further fluids were withheld for the subsequent 24 hours, during which period the serum sodium and chloride concentrations were observed to be maintained 5 to 10 milliequivalents above the upper limit of normal. The urinary output of chloride and, in some experiments, of sodium, was measured.

During the 24 hours following the injection, 2 control subjects, 1 with bronchial asthma, the other with idiopathic hypoproteinemia and edema, excreted 9.9 and 8.1 grams of chloride, expressed as sodium chloride. One patient with mild heart failure excreted 6.8 grams. Of 2 patients with severe congestive failure, 1 excreted 3.4 and 1.5 grams on different occasions, and the other 3.2 grams. One patient with severe heart failure and diminished renal function excreted 1.8 and 1.2 grams during different tests.

*Experimental Production of the Syndrome of Apparent Bundle-Branch Block With Short P-R Interval.* By J. SCOTT BUTTERWORTH and CHARLES A. POINDEXTER (introduced by Irving S. Wright), New York, N. Y.

The electrocardiographic syndrome of apparent bundle-branch block with short P-R interval has been reproduced in cats and dogs by the use of a special amplifier. The normal conduction system of the heart is short-circuited through the amplifier and a ventricular asynchronism is produced, giving the appearance of bundle-branch block. By reversing the direction of the current flow in this short circuit, supraventricular tachycardias can be produced.

The method is also adaptable to the study of other problems of cardiac physiology.

*A Study of the Time of Reaction of the Peripheral Blood Vessels in the Right Index Finger Tip and Right Second Toe Tip to Sensory Stimuli in Normal and Senile Subjects and Patients With Renal and Diencephalic Hypertension.* By G. E. BURCH, A. E. COHN, and (by invitation) C. NEUMANN, New York, N. Y.

The time of reaction of the peripheral blood vessels to sensory stimuli of constant intensity (bright light, sudden noise, pin prick, local heat, local cold and electric shock) was measured by recording the time necessary for the volume of these vessels to change on being stimulated. The stimuli were applied to the external surface of the left arm just above the elbow. The changes in volume were recorded by a sensitive pneumoplethysmograph. Measurements were accurate to 0.1 second. The indi-

viduals rested in the supine position in an air-conditioned room with fingers and toes at the level of their hearts. Observations were repeated several times.

The mean reaction time of the *finger* tip was 3.04 seconds in normal subjects; 2.90 seconds in patients with renal and diencephalic hypertension; and 4.01 seconds in senile subjects. The mean reaction time of the blood vessels of the *toe* tip of the normal subjects was 3.46 seconds; 3.21 seconds in patients with hypertension; and 4.07 seconds in the senile subjects. Clearly, the reaction times of the finger and toe tips of the normal and hypertensive subjects were essentially the same, while those in the senile subjects were significantly prolonged. Reference is made to the influence of psychological states.

*Capillary Blood Pressure In Man. Direct Determinations in the Digits of Subjects with Normal and Elevated Arterial Pressures.* By L. W. EICHNA (by invitation) and JAMES BORDLEY, III, Baltimore, Md.

Capillary blood pressure was measured in the digits by the direct microinjection method (Landis). In 47 hypertensive patients the capillary blood pressure varied within the same limits as in 60 subjects with normal arterial pressure. When the capillary blood pressure was measured in the same individual at different levels of arterial pressure, no correlation between the capillary blood pressure and the arterial pressure could be demonstrated, either (1) in 3 subjects in whom temporary hypertension was induced by the injection of paredrinol sulphate, or (2) in 4 hypertensive subjects whose arterial pressure fell to normal either spontaneously or after therapeutic surgical procedures.

During reflex vasodilatation and during the local vasodilatation of reactive hyperemia, the capillary blood pressure was approximately the same in subjects with elevated and with normal arterial pressure. During the local vasodilatation induced by histamine injected intradermally, the capillary blood pressure in hypertensive subjects exceeded slightly that in subjects with normal arterial pressure.

These studies indicate that in association with arterial hypertension there is, in the digits, an increased vascular resistance which is almost entirely pre-capillary in origin, and which may be released in some measure by histamine injected locally, but not by reflex vasodilatation or reactive hyperemia. These conclusions apply only to the digital circulation.

*Total Cardiac Vibrations Recorded by the Cathode Ray.* By WILLIAM B. KOUNTZ and (by invitation) JOHN R. SMITH, St. Louis, Mo.

Studies on cardiac vibrations over many years have embraced the limited range of audible frequencies comprising the heart sounds. An analysis of the total cardiac vibrations recorded by the cathode ray shows many variations of vibrations and also low-frequency vibrations (inaudible) which have never before been appreciated. Low frequency inaudible waves have been found in the tracing of normal persons, but are more frequently seen



in those with heart disease, particularly those with a weakened myocardium. In the latter, the low frequency deflections may assume excessive size; in some cases such changes antedate electrocardiographic alterations.

Low frequency systolic-diastolic waves of identical character may be obtained from the experimental heart under well-controlled conditions. The physiological factors which initiate the waves are not known, but appear to be subtle motions of the myocardium itself. Under experimental conditions the waves likewise increase in size as myocardial anoxemia progresses.

It appears that large low frequency waves in vibrocardiographic tracings from patients with heart disease may be reflections of myocardial disease. These may occur before other means of examination elicit the presence of a diseased heart.

*The Circulation in Traumatic Shock.* By N. E. FREEMAN and (by invitation) M. L. CULLEN and A. E. SCHECTER, Philadelphia, Pa.

Traumatic shock was produced in nine of eleven dogs and in five partially sympathectomized dogs. Local fluid loss was excessive in all instances. The earliest sign of shock as studied by us was a marked reduction of peripheral blood flow as measured in the dog's paw. Hemocentration was not a significant finding.

Shock was produced in fourteen of sixteen dogs with more severe trauma, but with restriction of local fluid loss. The earliest sign of shock was, as a rule, a marked reduction of peripheral blood flow. There was considerable hemoconcentration in comparison to the earlier experiments.

Blood volume determinations by the carbon monoxide method showed a substantial reduction of blood volume after trauma, and this reduction was only partially accounted for by local fluid loss.

Postmortem examinations suggest that at least part of the "lost" blood volume is to be found in the lumen and mucosa of the intestinal tract.

*Electrocardiographic Tracings in Normal and Abnormal Hearts Studied by the Method of Schlesinger.* By P. M. ZOLL and E. SPIEGL (by invitation) and H. L. BLUMGART, Boston, Mass.

A comparison of the electrocardiographic tracings and the postmortem findings was made in 201 cases in which complete pathologic study of the myocardium and coronary arteries was made, utilizing the Schlesinger method of injection. Measurement of the collagen content of the myocardium was also done in many instances.

Patients with normal hearts frequently showed electrocardiographic changes usually considered abnormal, such as delayed A-V conduction, axis deviation, deviations in the Q, S, T and ST intervals, notching of QRS, and even negative monophasic QRS. These abnormalities were more frequently observed when the tracings were taken during acute non-cardiac illness, in which instances they may be ascribable to myocardial ischemia of non-cardiac origin. These observations indicate that the limits of the

so-called "normal electrocardiogram" must be extended and that deviations now considered pathologic are compatible with the finding of a normal heart on postmortem examination.

In the absence of myocardial infarction or extensive fibrosis, patients with old coronary occlusions of the main coronary arteries may show no electrocardiographic differences from those tracings taken in patients with normal hearts. This is in accord with previous clinical and pathologic studies by us which show that the collateral circulation may compensate fully for complete occlusion of a coronary artery.

The pathologic findings of acute anterior and posterior wall infarction were closely reflected by typical and progressive electrocardiographic patterns. Single tracings, supposedly characteristic of acute infarction, were seen, however, during episodes of "acute coronary failure" (i.e., prolonged myocardial ischemia without infarction) with a rapid return to normal after the attack. ST and T wave deviations similar to those found in acute infarction were also seen in acutely ill patients with normal hearts. The fact that such changes may be witnessed secondary to disease of non-cardiac origin is of considerable importance in questions of differential diagnosis between cardiac and non-cardiac pain in seriously ill patients. Unless it is recognized that such changes may occur in the absence of cardiac disease, an erroneous diagnosis of myocardial infarction may be made.

*Studies on the Effect of Cold and Heat in Patients With Angina Pectoris.* By A. S. FREEDBERG, E. D. SPIEGL (by invitation) and J. E. F. RISEMAN, Boston, Mass.

The increased frequency of attacks of angina in daily life when patients exercise in the cold is well recognized. This clinical fact has been utilized in the laboratory to precipitate attacks of angina at will by having the patients exercise in a room kept at a constant cold temperature (45 to 55° F.). This "Standardized Exercise Tolerance Test" has been of value in diagnosis and in measuring objectively the response to treatment. This communication presents evidence concerning the manner in which cold operates to facilitate the production of angina.

Nine of a series of 20 patients with angina pectoris were able to do an appreciably greater amount of work when exercising at ordinary room temperature (75 to 80° F.) than when exercising in the cold temperature room. When these patients exercised at room temperature, while holding an ice cube in one hand, angina was precipitated by the same minimal amount of work which induced an attack in the cold temperature room. Conversely, when these patients immersed their hands in hot water (110° F.), or strapped a hot water bottle to the body before exercising in the cold temperature room, they were able to do as large an amount of work as was possible when exercising at room temperature. No appreciable differences in the heart rates, blood pressures or electrocardiograms of these patients were observed under the various conditions of temperature and exercise used in these studies. One patient receiving digitalis developed



anoxemic changes in the electrocardiogram shortly after exposure to cold without exercise. Each of these 9 patients showed a favorable therapeutic response to vasodilating drugs.

The remaining 11 patients showed no appreciable difference in the amount of work necessary to induce angina at room temperature or in the cold. These patients with a fixed exercise tolerance showed no appreciable therapeutic response to vasodilating drugs.

To summarize, there are two groups of patients with angina on effort: (1) those in whom the exercise tolerance is unaffected by moderate changes in temperature as used in these studies; (2) those in whom the exercise tolerance is definitely decreased by cold. The reduction in exercise tolerance in a cold room may be duplicated by exercise in a warm room if the patient holds ice in his hand. These observations accord with the concept that coronary artery vasomotor changes, probably reflex in origin, exert a contributory influence in angina pectoris and correspond to the clinical observation that patients with angina pectoris, even though warmly clothed, suffer attacks particularly on cold days. These observations are likewise helpful in learning whether a patient will benefit by the prophylactic administration of vasodilator drugs.

*Dynamics of Blood Flow in Thrombo-angiitis obliterans.*

By M. LANDOWNE\* (by invitation) and L. N. Katz,† Chicago, Ill.

Studies in blood flow in thrombo-angiitis obliterans were made by a critically evaluated plethysmographic method. Fifty-two experiments are reported on the horizontal foot-leg of 6 ambulatory patients, 30 to 45 years of age. The recorded "initial" resting flows were compared with "maximal" resting flows; the latter being induced by heat (plethysmographic temperatures of 42 to 44° C. for 30 minutes), by reactive hyperemia (cuff inflated to 200 to 300 mm. Hg about the thigh for 5 to 15 minutes) and/or by both together.

Recorded "initial" resting flows showed little fluctuation. The values (1.2 to 7.5 cc. per minute per 100 cc. limb) were within the range of control observations on 18 normal subjects.

"Maximal" resting flows (2.5 to 7.5 cc. per minute per 100 cc. limb) recorded with the dilating procedures used in these patients showed no significant increases over the "initial" level, unlike controls in whom marked increases in flow were consistently demonstrated (7.3 to 22.6 cc. per minute per 100 cc. limb). The slight increases noted occurred in the patients with milder involvement.

Since in this condition the presumed site of disease is not in the smallest blood vessels, it is assumed that these tiny vessels are capable of being dilated if not already dilated. The results are consequently interpreted as offering a measure of the effective obstructive resistance of the diseased larger vessels, which limits the amounts of blood flowing into the limb per unit of time (for a given cardiac output and central blood pressure). This does not ex-

clude the possibility of change in distribution of blood within the limb following these dilating procedures.

*Changes in Cardiac Output, Fluid Volume, and Kidney Function on Recovery from Congestive Heart Failure.*

By WALTER H. PRITCHARD, WILLIAM B. SEYMOUR, and L. P. LONGLEY (introduced by J. M. Hayman, Jr.), Cleveland, O.

Cardiac output, blood volume, interstitial fluid volume, plasma proteins, etc., and inulin, urea, and phenol red clearances were determined on 7 patients which congestive heart failure, and again when maximal compensation was secured by rest, digitalis, and other therapy. Cardiac output determined by a modification of Bazett's method increased 13 to 85 per cent in 6 patients; in the seventh there was a decrease of 23 per cent at a time when he was showing evidence of digitalis intoxication. Venous pressure, blood volume (Evans Blue method) and extracellular fluid (thiocyanate method) value decreased in all cases. The change in the last of these approximated the loss of body weight. While the concentration of serum proteins rose, the total serum protein in circulation fell by 9 to 48 grams in 6 patients; loss of serum albumin accounted for approximately 2/3 of the total. Phenol red clearance varied with cardiac output, as did inulin with one exception. The fraction of the cardiac output represented by the inulin clearance was quite constant. Urea clearances were more variable.

*The Form of the Dog's Precordial Electrocardiogram in Bundle-Branch Block Complicated by Anterior Infarction.\**

By HERMAN ERLANGER, FRANCIS F. ROSENBAUM, NELSON COTRIM (by invitation) and FRANKLIN D. JOHNSTON and FRANK N. WILSON, Ann Arbor, Mich.

Dogs of medium or large size were fully anesthetized and the heart was exposed under aseptic conditions. Either the right or left branch of the His-bundle was then cut and the anterior descending branch of the left coronary artery was ligated at a high level. If the heart survived these procedures, the chest was fully restored, and the animal was allowed to recover. One to seven weeks later an extensive electrocardiographic study, including multiple precordial leads and leads from the surface of the exposed heart, was made. Of 16 animals studied in this way, 3 had right branch block without infarction; 3 had right branch block plus infarction; 1 had left branch block without infarction, and two had left branch block plus infarction. The observations made justify the following conclusions: In canine bundle-branch block, precordial leads yield curves which are similar in every respect to those obtained in human branch block of the same variety. In canine right branch block complicated by infarction, precordial leads yield QRS-complexes of characteristic form; large Q-waves are present in leads from points

\* An experimental study carried out with the help of a grant to F. N. Wilson from the Horace H. Rackham School of Graduate Studies.

\* Emanuel Libman Fellow.

† Aided by the A. D. Nast Fund for Cardiac Research.

overlying the infarcted region and late R-waves occur in leads from the right side of the precordium. In canine left branch block complicated by infarction, the precordial curves are difficult to distinguish from those obtained in uncomplicated left branch block.

#### *Dorsolumbar Sympathectomy for Essential Hypertension:*

*Results and Bearing on Etiological Considerations.* By ROBERT STERLING PALMER and (by invitation) REGINALD H. SMITHWICK, Boston, Mass.

A theory for the etiology of essential hypertension in man must embrace certain proved and a few probable facts:

1. Perturbation of the renal circulation by compression of the main renal arteries in experimental animals apparently alters a pressor-depressor hormonal balance, resulting in a pressor effect and systemic arterial hypertension.

2. There is no pathognomonic pathological change in the arterioles in the terminal stage of continued arterial hypertension, but there is a pathognomonic localization of such change when of marked degree, namely the renal arterioles.

3. Congenital hypoplasia, metabolic faults, infection or stases may cause the perturbation of the circulation or explain the specific localization of arteriolar disease in about 20 per cent of patients with continued arterial hypertension. In 80 per cent of patients with continued arterial hypertension there is no such explanation of the specific localization.

4. Partial or total sympathectomy does not prevent or modify experimental hypertension which follows the irreversible narrowing of the main renal arteries by application of the Goldblatt clamp, but partial sympathectomy, sufficient to denervate the kidneys, adrenals and the splanchnic bed, significantly lowers the blood pressure in a majority (60 per cent) of patients with so-called essential hypertension.

There appear to be only two explanations of the last fact: (a) Sympathectomy modifies the effect of organic renal constriction in man, contrary to the experience with experimental animals. Due to man's habitually upright position, according to this possibility, splanchnic denervation causes venous pooling, diminished return of blood to the heart, decreased output which lowers the head of the pressure component of arterial hypertension, and reduction of the blood pressure. (b) The alternative explanation for the favorable effect of sympathectomy in patients with continued arterial hypertension is that an overactive sympathetic vasoconstrictor outflow, or an abnormal response to a normal vasoconstrictor outflow, is interrupted, thus significantly modifying the pressor responses to posture, cold, mental stress and physiological emergencies.

The venous pooling with consequent inadequate output does not explain the favorable effect obtained in the 12 per cent of cases in which a sustained and marked improvement in the blood pressure has followed supradiaphragmatic sympathectomy (Peet) without the relative postural hypotension noted following dorsolumbar sym-

pathectomy (Smithwick). The latter operation was undertaken in order to insure complete denervation. The relative postural hypotension may be a by-effect rather than a direct cause of the 60 per cent favorable results found thus far, since, as the relative postural hypotension becomes less marked, the favorable effect on the level of the blood pressure is maintained or even enhanced. We venture to suggest that it is a special condition in man, namely, his habitual upright posture, that places the circulation of the kidney at the mercy of the splanchnic bed, the function of which is the maintenance of the circulation to functioning tissue in the upper extremities, heart, lungs and especially the head. Furthermore, in the upright posture man is under the stresses of recurring emergencies. Pressor responses in certain human strains are unusually violent. May it not be that this overactive vasoconstrictor mechanism (or overactive response to a normal mechanism), operating over a long period of time in susceptible individuals, induces the specific localization in or just afferent to the nephron? This may at first be a functional vasoconstriction, leading to organic change, and finally an organic plus functional vasoconstriction. Such an etiological train of events would seem to fit more closely the observed clinical course of essential hypertension in man than the supposition of an unexplained chance localization of organic arteriolar disease, which in turn disturbs the pressor-antipressor hormonal balance in the kidneys.

*Measurements of the Circulation in Dogs with an Interventricular Septal Defect.* By EUGENE C. EPPINGER and ROBERT E. GROSS (introduced by C. S. Burwell), Boston, Mass.

A communication (4 to 6 mm. in diameter) between the ventricles of the heart was produced in 5 dogs. Before establishing the communication, determinations of the cardiac output were made by the direct Fick method. After operation, the pulmonary blood flow was determined by the comparison of blood from the aorta with that from the pulmonary artery (aorta-pulmonary artery oxygen difference) and the peripheral blood flow by a comparison of the blood in the aorta with that in the right auricle (aorta-right auricle oxygen difference).

These studies showed that such a communication between the ventricles leads to:

1. A higher oxygen content in the blood of the right ventricle than in the right auricle, indicating a shunt of blood from the left to the right ventricle. This leak amounted to 30 to 50 per cent of the output of the left ventricle.
2. An increase in the amount of blood flowing through the pulmonary artery and a decrease in the peripheral blood flow.

Since the blood shunted from the left to the right ventricle promptly returns to the left ventricle via the pulmonary circulation, the output of the left ventricle continues to be the same as that of the right.

In these animals the total output of both ventricles is greater after the production of the septal defect than be-

fore; therefore, the lesion imposes a significant burden on the heart and represents a considerable amount of wasted cardiac effort. X-rays of the heart taken before and after producing the lesion have shown that the presence of such a septal defect leads to a marked increase in the size of the right ventricle.

The following observations in one dog are cited as an example:

Cardiac output before operation ..... 3.15 liters per minute

After operation:

Pulmonary blood flow ..... 4.07 liters per minute

Peripheral blood flow ..... 2.63 liters per minute

Amount of shunt ..... 1.44 liters per minute

*A Simple Method for the Bioassay of Renin.* By OTTO SCHALES and FLORENCE W. HAYNES (introduced by G. P. Grabfield), Boston, Mass.

In connection with work on hypertension it was of importance to have a simple method for testing a large number of extracts for their renin content. Rabbits were used for the tests as their blood pressure is conveniently measured in the ear artery by a membrane manometer. A test procedure was standardized which, despite its simplicity, gives reliable and reproducible results. The extracts were injected intravenously in the right denervated ear. For the assay of each extract 4 animals were used and amounts were injected which would cause a rise in blood pressure of 20 to 40 mm. mercury.

From the data on 4 animals there was calculated the amount of renin (as micrograms of nitrogen) which had to be given per kilogram of rabbit to cause an average rise in blood pressure of 30 mm. We call this amount one rabbit unit.

If  $S$  = sum of renin per kilogram (in micrograms of nitrogen) in 4 rabbits and  $B$  = sum of rises in blood pressure in 4 rabbits, then  $\frac{S}{B} \times 30 = 1$  R.U.

This method for the bioassay of renin makes it possible to give data concerning yield and degree of purity for each step of the various chemical procedures involved in the preparation of renin.

*Electrocardiographic and Radiological Studies of the Heart During Normal Pregnancy.* By A. GERSON HOLLANDER (introduced by J. Hamilton Crawford), New York, N. Y.

During the course of pregnancy, especially when murmurs are present, the question arises as to whether organic heart disease is present. X-ray changes are sometimes found which seem to support such a diagnosis. In order to study the x-ray variations which occur in the heart during normal pregnancy, eleven cases with no suspicion of heart disease have been followed throughout pregnancy and postpartum. In addition, a series of twenty individuals who had suspicious findings during pregnancy, but who postpartum proved normal, was analyzed. Postero-anterior, left and right oblique views

with the barium-filled esophagus were taken. The most common finding in both series was an invasion of the anterior wall of the esophagus, usually with no displacement of the esophagus as a whole. Another change less frequently present was prominence in the region of the pulmonary conus and pulmonary artery and, on rare occasions, elevation of the left bronchus. These changes seldom took place simultaneously.

Twenty-two normal women were followed throughout pregnancy with serial electrocardiograms, leads I and III being taken synchronously. The common alterations which took place were the development of a deep Q wave and a negative T in lead III. There was a gradual shift of the electrical axis to the left of 20 to 30 degrees. The axis, however, at no time exceeded the recognized normal limits.

*A Piezoelectric Manometer for Various Clinical Studies of the Form and Velocity of the Intraesophageal Pressure Pulses.* By JAN NYBOER (introduced by Herman O. Mosenthal), New York, N. Y.

A successful attempt was made to substitute an electrical method (J. Clin. Invest., 1938, 18, 511) for the conventional mechanical one of an optical membrane-manometer as the terminal pressure instrument for intraesophageal pulse studies. In this device, a small rubber balloon was connected by a (Number 16 Fr.) soft rubber esophageal catheter to a metallic aneroid on which was mounted a wire leading to a piezo-crystal unit. The potentials generated from the crystal by the undulating pressures in the aneroid were recorded by a string galvanometer controlled by a vacuum-tube amplifier. Simultaneous tracings of pressures by both methods proved an equivalent accuracy but greater convenience and increased sensitivity of the electrical device was noted as compared with the mechanical one.

For purposes of study of the relative velocity and direction of the complicated pressure changes during the cardiac cycle, the piezoelectric potentials may be electrically differentiated by the method described elsewhere (J. Clin. Invest., 1940, 19, 963).

For increased accuracy in reference to a given retrocardiac level, simultaneous esophageal electrocardiograms were made from an electrode fixed at the tip of the catheter near the esophageal balloon. Tracings were taken in 2.5 cm. steps between the lower end of the esophagus and a position opposite the aortic arch. These pressure pulses confirm in part their recent description by Taquini (Am. Heart J., 1940, 20, 129) and suggest their probable usefulness in the study of the normal and abnormal heart. An attempt was made to evaluate the juxta-ventricular esophageal pulsation in cases of posterior myocardial infarction.

*The Production of Hypothyroidism in Rabbits by Injections of Thyroglobulin. Its Relation to Refractoriness.* By J. LERMAN, Boston, Mass.

The demonstration of thyroglobulin antibodies by Hektoen and Schulhof (J. A. M. A., 1923, 80, 386)

raised the question, "Are these antibodies antimetabolic (antihormones)?" In 5 out of 7 rabbits immunized with human thyroglobulin there developed some degree of refractoriness to subsequent intraperitoneal injections of thyroglobulin. The negative results of other investigators (Schulhof, *Am. J. Physiol.*, 1930, 93, 175 and Rosen and Marine, *Am. J. Physiol.*, 1937, 120, 121) are, I believe, due to technical difficulties.

Another important attribute of thyroglobulin antibodies is that they are "hormone-specific" rather than "species-specific."

These two effects should theoretically lead to inhibition of the immunized animal's own thyroid. In fact, 7 of 8 rabbits immunized with human thyroglobulin over a long period of time (6 months or more) developed metabolic rates of minus 20 to minus 35 per cent below normal; they behaved in other respects like myxedematous animals. Another animal reached a level of minus 14 after 5 months of immunization. The findings of Morgan and Ivy (*Proc. Soc. Exper. Biol. and Med.*, 1934, 31, 1139) are, in some respects, similar. They produced "cretin-like" rabbits by injection of serum from chickens which had been immunized with a crude extract of thyroid.

Inasmuch as one of our main interests is the treatment of human hyperthyroidism, and since the above results suggested a possible mode of controlling the disease, we immunized one patient suffering from Graves' disease with sheep thyroglobulin for 3 months. It was impossible to obtain a high antibody titer in the serum of this patient, and the clinical results were entirely negative. Further trials of this sort are indicated.

*Pitressin in Oil. Prolonged Antidiuretic Effect in Experimental and Clinical Diabetes Insipidus.* By D. J. STEPHENS, Rochester, N. Y.

The recent literature contains references to prolongation of the antidiuretic effect of posterior pituitary extracts emulsified in oil and beeswax by the subcutaneous implantation of posterior pituitary powder, by the injection of pituitrin and posterior pituitary extracts in the presence of zinc salts and by the administration of a suspension of pitressin in oil. This communication reports the results of a study of the effect of pitressin in oil in two dogs with experimental diabetes insipidus and in 7 patients with diabetes insipidus. Urine was collected in 4-hour periods and the volume, specific gravity, and, in some instances, chloride concentration were determined before and after the subcutaneous administration of an aqueous solution of pitressin and of a suspension of pitressin tannate in peanut oil. The duration of the antidiuretic effect of the largest tolerated doses of aqueous pitressin (10 to 20 pressor units) was from 4 to 8 hours. Single doses of 1 cc. of pitressin in oil (4 pressor units) exhibited antidiuretic effect of from 24 to 96 hours in duration. The maximum effect of pitressin in oil on urine volume and specific gravity was greater than the maximum effect of the largest tolerated doses of pitressin in aqueous solution. No local or general reac-

tions were encountered after the administration of pitressin in oil.

Considering both intensity and duration of antidiuretic effect, pitressin in oil is at least 15 times as effective as aqueous pitressin. In patients with diabetes insipidus, not only is the number of hypodermic injections greatly reduced, but symptoms, urine volume, and specific gravity are more consistently and satisfactorily controlled by pitressin in oil than by any other method of treatment available at the present time.

*Clinical and Biochemical Studies of Female Hirsutism and Virilism with Special Reference to Differential Diagnostic and Prognostic Significance of Urinary Ketosteroid Excretion.* By HARRY B. FRIEDGOOD (introduced by James P. O'Hare), Boston, Mass.

Clinical and biochemical observations were made on 10 normal women, on 65 patients with various types of hirsutism and virilism not attributable to neoplasm, and on 2 cases of virilism due to malignant corticoadrenal tumor. Included among the clinical studies were the following: (1) Precipitating factors related to the onset of hypertrichosis (*e.g.*, menarche, menopause, pregnancy, amenorrhoea, etc.); (2) course of development, extent and distribution of various types of hypertrichosis; (3) physiological significance of therapeutically-induced and spontaneous improvement in hypertrichosis.

Biochemical studies were concerned with establishing the range of 24-hour ketosteroid excretion in various circumstances. The ketosteroid output of normal women and of hirsute patients fell within the same range; that of virilistic women was generally outside the upper limits of normal, although these values overlapped occasionally. Ketosteroid determinations gave reliable help in the differential diagnosis between virilism due to corticoadrenal tumor and that not attributable to neoplasm. Quantitation of the ketosteroids was also of aid in determining prognosis, inasmuch as it led to the prediction of post-operative recurrence of a corticoadrenal tumor several months before this was suspected clinically.

The marked increase in urinary ketosteroids which is characteristic of tumor-induced virilism is due to the excretion of abnormally large amounts of dehydroisoandrosterone, which ordinarily constitutes only a small fraction of the androgen content of urine. Dehydroisoandrosterone is determined quantitatively, therefore, for its diagnostic value whenever corticoadrenal tumor is the suspected cause of virilism.

*The Relation of Dark Adaptation (Determined by the Hecht Adaptometer) to Blood Carotene and Blood Vitamin A Levels.* By E. WHITE PATTON and W. R. SUTTON (by invitation) and JOHN B. YOUNG.

Using the Hecht adaptometer, the rod threshold for light perception was determined in approximately 50 individuals. In each individual, the threshold was obtained both with and without previous light adaptation. When light adaptation preceded dark adaptation, the technic described by Hecht was used. When no previous light

adaptation was employed, the subject was allowed to dark adapt for 20 minutes and the threshold was then measured. The correlation of the rod thresholds obtained by the two technics was quite satisfactory. The group included individuals with normal and elevated thresholds, and the individuals varied in age from 8 to 72 years.

Using the threshold obtained without previous light adaptation, a comparison was made between the rod threshold, the blood serum vitamin A, and the serum carotene levels in approximately 400 individuals. Each subject was examined twice, once in the fall and again in the spring. The average dietary intake of the entire group was decidedly more adequate in vitamin A and carotene at the time of the fall examination. Considering the entire group, little direct correlation could be discerned between the rod threshold levels and the blood serum vitamin A or carotene levels. There was a striking difference in the incidence of abnormal serum vitamin A and serum carotene levels in the two examination periods. On examination in the spring, the level of serum vitamin A and carotene in the blood was low in a large number of the individuals. When the same individuals were reexamined in the fall, the majority of them were found to have normal levels. No significant difference was found in the number of abnormal adaptometer thresholds occurring in the two examination periods.

Further work is now in progress to evaluate the relationship of the dietary intake of vitamin A and carotene to these procedures, and other factors such as age, etc., which may influence them are being studied.

*Hormonal Factors Influencing Skeletal Growth.* By WILLARD O. THOMPSON, and (by invitation) NORRIS J. HECKEL and RICHARD M. MORRIS, Chicago, Ill.

Studies in man during the past several years appear to support the following conclusions:

1. The growth and configuration of the skeleton are under hormonal control.
2. The thyroid and the pituitary appear to affect primarily the length of the skeleton. There is some evidence that the thyroid affects the growth of bone by stimulating the production of growth principle in the anterior lobe of the pituitary.
3. The gonads through their production of sex hormones affect the proportions of the various parts of the skeleton, as well as its length. Thus it may be shown that:

(a) In men and women whose gonads have not functioned during the period of skeletal growth the trunk is usually very short in proportion to the extremities, the total height of the body being within normal limits. This disproportion may be in part the result of failure of epiphyseal growth in the vertebrae which occurs at the time of puberty.

(b) In young boys the administration of either chorionic gonadotropin or male sex hormone will not only cause stimulation of genital growth but will also cause

the skeleton to grow in length at an abnormally rapid rate and will cause the trunk to become long in proportion to the extremities. Whether or not premature stimulation of genital growth causes premature closure of epiphyses is still a debatable point. It would appear that closure of epiphyses is dependent upon a certain amount of aging in the bone itself.

(c) In pituitary dwarfs with hypogonadism in whom the epiphyses are open, administration of the male or female sex hormone, depending upon the sex of the individual, may result in a great increase in length of the skeleton and a proportionately greater increase in the length of the trunk than of the extremities.

4. The influence of hormones is modified by hereditary and nutritional factors which affect bone directly.

The growth of the skeleton must, therefore, be thought of as a complex process which involves a delicate balance between hormonal, hereditary and nutritional factors.

*Studies of Lymph in the Adrenalectomized Dog.* By OLIVER COPE, ADDISON G. BRENIZER, JR. (by invitation), HUGO POLDERMAN (by invitation) and HENRY K. BEECHER, Boston, Mass.

Using local procaine anesthesia, the cervical lymphatic trunks were cannulized in trained normal and adrenalectomized dogs in various stages of insufficiency. The flow of lymph was stimulated either by rhythmic motion of the head or gentle massage. The lymph was compared with the serum of blood samples removed during collection; the proteins, amylase activity, NPN, Na, K, Ca, total base, Cl, CO<sub>2</sub>, PO<sub>4</sub>, pH, sugar and hematocrit were measured. The arterial blood pressure and plasma and extracellular fluid volumes were determined.

The outstanding change in lymph encountered was a rise in protein from 2.0 to 2.5 per cent in the normal to 2.7 to 5.0 per cent in the insufficient dogs. The serum protein did not show a corresponding rise. The amylase activity\* of lymph rose parallel to the activity of serum but was lower in lymph than in serum in both the normal and insufficient animal. The electrolytes, NPN and sugar followed closely the levels in the blood of both the normal and insufficient animal. The volume of lymph collected was increased in the insufficient dog. The reported decreased plasma and increased extracellular fluid volumes were confirmed. The findings suggest increased capillary permeability in adrenal cortical insufficiency in the dog.

*Measurements of the Velocity of Sound in the Human Body and the Value of this Quantity in the Design of Stethoscopes.* By FRANKLIN D. JOHNSTON and PAUL S. BARKER, Ann Arbor, Mich.

When a sound wave passes from a dense into a rare

\*Cope, Oliver, Kapnick, Israel, Lambert, Adrian, Pratt, T. Dennie, and Verlot, Max G. Endocrine function and amylase activity. II. Changes in activity of blood serum amylase in response to changes in adrenal cortical function in the dog and rabbit. *Endocrinology*, 1939, 25, 236.

medium much of the energy in the wave is reflected at the boundary so that very little is transmitted to an observer in the rare medium (air). The physical characteristics of the two media will determine the efficiency with which such transmission takes place. Thus, sounds arising in water are very poorly heard in air since only 0.12 per cent of the energy in the wave striking the water surface passes into the air. Similar considerations apply to sounds originating within the human body.

A method for estimating the average velocity of transmission of sound through the human chest has been developed and, when this velocity is multiplied by the mean density of the thoracic structures, a very useful quantity—the specific acoustic resistance—is obtained. Knowing this, it can be shown that less than 1 per cent of the sound energy arising inside the body passes into the air.

A stethoscope serves to increase greatly this transfer of energy by acting as an acoustic matching transformer between the body and the air, and the form of the end-piece necessary to achieve this end most efficiently can be calculated from the data at hand.

*The Oxygen Consumption of the Intact Mammalian Heart.* By G. V. LEROY (introduced by Richard B. Capps), Chicago, Ill.

The oxygen consumption of the heart of the intact dogs

was studied by means of a modification of the direct method of Harrison, Resnik and Friedman. Two types of anesthesia were employed—nembutal and dial. The data are compared with those of Harrison, *et al.*, who used "appropriate amounts" of morphine. In general, in this type of experiment the oxygen consumption and the mechanical efficiency of the heart vary inversely as the depth of the anesthesia. The dogs of Harrison, *et al.*, who were not submitted to a surgical procedure displayed the highest values of oxygen consumption and efficiency; the next highest values were in our nembutal group; the next highest were found in the dogs of Harrison, *et al.*, who had been operated upon; and the lowest values were from our dial group. Values for these same factors obtained from heart-lung preparations are generally lower because of the trauma encountered. Thus, anesthetic agents demonstrably influence cardiac energetics in an unfavorable manner.

An observation of considerable importance, and one not emphasized heretofore, is the low oxygen saturation of coronary venous blood. This value averaged 20 per cent of arterial in our series, and was lower than one had imagined it to be. A simple calculation will demonstrate that even a normal heart cannot long tolerate a reduction of the coronary flow by more than 20 per cent without suffering myocardial ischemia.



# INFLUENCES OF ERYTHROCYTES AND OF LEUKOCYTES ON STABILITY AND TRANSFER OF ASCORBIC ACID IN HUMAN BLOOD<sup>1</sup>

By MARTIN HEINEMANN

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(Received for publication April 22, 1941)

Hitherto, in investigations of the stability and distribution of ascorbic acid in blood (1, 2), no attempt has been made to distinguish the relative activities of erythrocytes and leukocytes. These two groups of cells, however, differ with respect to development, structure, function and fate. It therefore seemed desirable to study the influence on and the reaction to the serum ascorbic acid of each type of cells separately. The results of such an investigation are here reported. Some observations on the influence of composition and concentration of serum protein on distribution and stability of serum ascorbic acid are added.

## METHODS

Blood from healthy human subjects only was used. For reasons set forth previously (1), no anticoagulant was added, but freshly drawn blood was defibrinated by stirring with a glass rod. In defibrinated blood, leukocytes cannot be separated from erythrocytes as distinctly as in oxalated blood. Therefore, a compromise was worked out on the following basis: Instead of studying red blood corpuscles and leukocytes separately, *i.e.*, each without the presence of the other, erythrocytes with and without leukocytes were observed. Once the effect of the presence of erythrocytes became known, differences in reactions noted with erythrocytes plus leukocytes were ascribed to the presence of the latter.

Defibrinated blood was transferred to ordinary conical 15 cc. centrifuge tubes (Pyrex glass) which were stoppered and kept for 1½ hours at about 25° C. During this time of slow spontaneous sedimentation, leukocytes remain in the upper layer. Thereafter the tubes were centrifuged at moderate speed for 20 minutes. After carefully removing the supernatant serum,<sup>2</sup> a semi-capillary pipette was introduced to the bottom of the centrifuge tube and approximately four-fifths of the cells were transferred to a flask by means of suction. This portion of cells was found to contain no leukocytes, or almost none. Serum was added in amounts proportional to the number of erythrocytes desired. The re-

maining one-fifth of cells was gently shaken with about twice its volume of serum in order to suspend the layer of cells adherent to the wall of the centrifuge tube.

## Determination of ascorbic acid

In whole blood, ascorbic acid was determined by the method of Emmerie and van Eekelen (3), in plasma by that of Mindlin and Butler (4).

For the latter procedure, if applied to serum obtained from defibrinated blood, it has been shown previously (1) that a stable color with dichlorindophenol is only developed if the final reaction of the filtrate is so adjusted that 0.215 cc. of 0.1 N NaOH will bring it to the neutral point with phenolphthalein as indicator. From sera with normal protein content filtrates of this normality can be attained when 0.515 N HPO<sub>3</sub> is used for deproteinization. Since one volume of serum is diluted with one volume of distilled water before two volumes of acid are added, the latter becomes diluted once, reducing its normality to  $\frac{0.515}{2} = 0.2575$  N. With normal sera, then,  $0.2575 - 0.215 (= 0.0425)$  constitutes the decrease in normality due to the combination of HPO<sub>3</sub> with protein. If ascorbic acid is to be measured in sera with abnormal protein concentrations, as in some of our experiments, this decrease in normality has to be measured in advance in order to obtain filtrates of 0.215 N. For this purpose, serum is precipitated with 0.515 N HPO<sub>3</sub>, and the normality of the filtrate (A) is measured by titration with 0.1 N NaOH. It was found experimentally that the following equation can be applied:

$2(0.2575 - A + 0.215) = 2(0.4725 - A) = \text{normality required for precipitation.}$  Transfers of ascorbic acid from serum to cells were estimated as in previously described experiments (2).

Different concentrations of protein in the same serum were obtained by ultracentrifuging for 2 hours at 45,000 r.p.m. (142,000 g.), the tubes being held at an angle of 25° to the vertical.

Attempts to study serum substrates and filtrates by using Lavietes' method of ultrafiltration (5) were unsuccessful because contact with mercury causes significant destruction of ascorbic acid, even under anaerobic conditions and at 7° C.

## EXPERIMENTAL RESULTS

*Effect of erythrocytes and of leukocytes on stability of serum ascorbic acid.* Serum separated

<sup>1</sup> Aided by a grant from the John and Mary R. Markle Foundation.

<sup>2</sup> Serum thus removed must be centrifuged again since it may still contain a few cells.



at once from defibrinated blood was continuously agitated at about 25° C. and at 37° C. in an atmosphere of air. Under such conditions, ascorbic acid was observed to be destroyed in 9 different separated sera at a rate of from 5 to 10 per cent of the original per hour. This is about the same rate reported by Kassan and Roe (6). Figure 1*A* depicts one experiment representative of 8 with essentially identical findings. The presence of

erythrocytes invariably protected ascorbic acid in serum, whereas the presence of leukocytes enhanced the rate of destruction.

*Transfer of added ascorbic acid from serum to erythrocytes and to leukocytes.* Figure 1*B*, representative of 5 experiments, demonstrates that (1) no significant decrease in the concentrations of ascorbic acid in true serum was noticeable with erythrocyte counts varying from 3.7 to 4.4 mil-

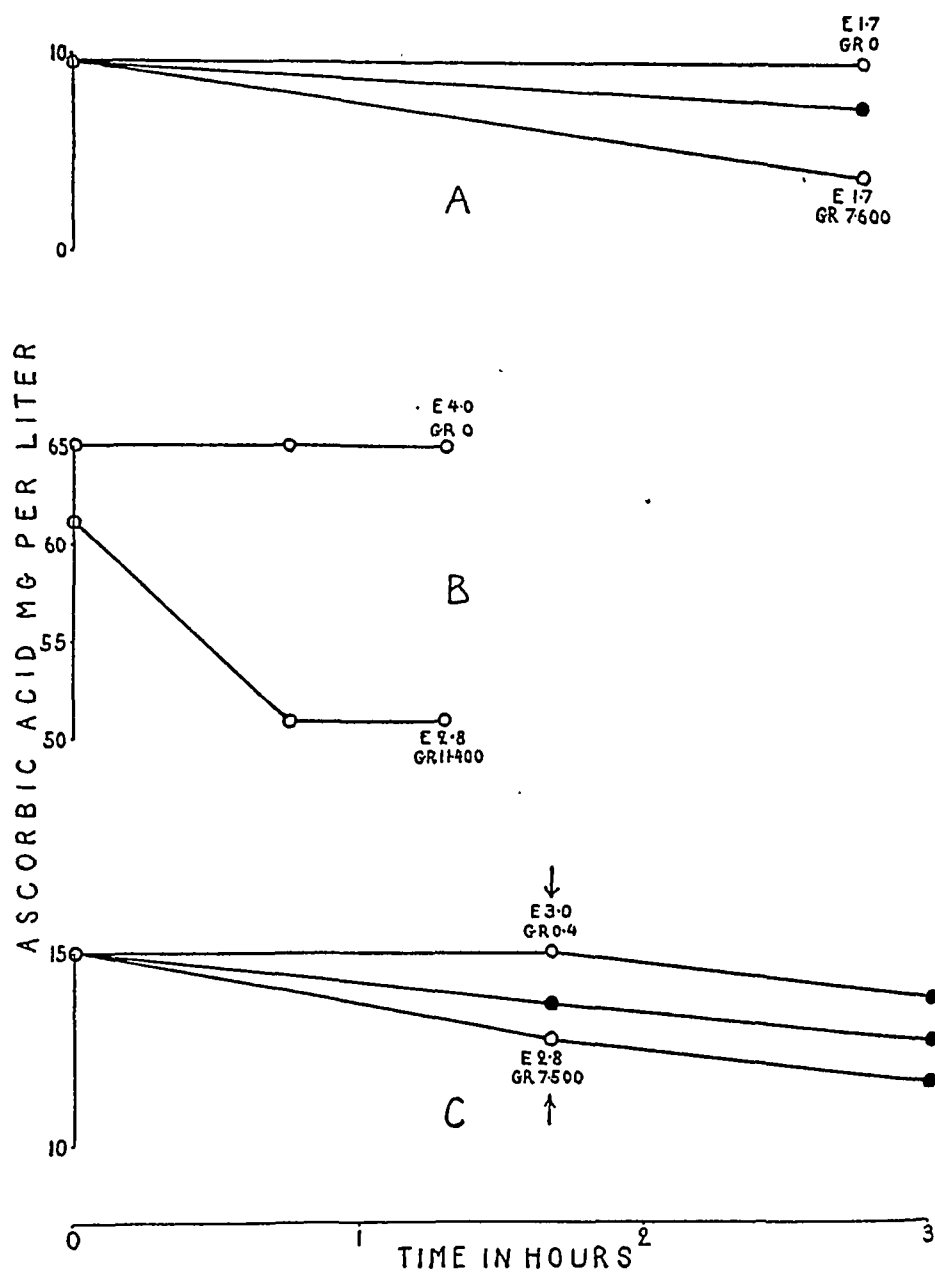


FIG. 1. STABILITY AND TRANSFER OF ASCORBIC ACID IN BLOOD CONTAINING DIFFERENT TYPES OF CELLS

Solid circles indicate sera separated from cells at zero time, open circles indicate sera removed immediately before determinations. All experiments were carried out with continuous agitation; *A* and *C* at room temperature in an atmosphere of air; *B* under nitrogen at 37° C. "E" and "GR" = abbreviations for erythrocytes (in millions) and leukocytes (in thousands).

lions per cubic millimeter and (2) when leukocytes were also present, transfers of ascorbic acid regularly occurred and, as previously observed, were self-terminative in about 30 minutes. The amounts transferred were proportional to the number of leukocytes.

*Effects of different protein concentrations on distribution and stability of ascorbic acid in serum.* Table I presents concentrations of ascorbic acid in

TABLE I

*Distribution and stability of ascorbic acid in serum when protein concentrations were changed by ultracentrifuging*

	Upper layer	Middle layer	Lower layer
Total protein, per cent. ....	4.46	5.54	7.60
Albumin, per cent. ....	3.30	4.26	4.71
Globulin, per cent. ....	1.16	1.28	2.89
Ascorbic acid, mgm. per liter, initial. ....	13.2	13.4	12.8
After one hour at 28° C. in air. ....	11.8	11.6	11.6

\* To 50 cc. of serum from defibrinated blood one cc. 1.5 M phosphate buffer pH 7.3 was added in order to maintain identical pH in all layers.

different layers obtained from the same serum by ultracentrifuging. It also shows the rate of deterioration of ascorbic acid in each layer when exposed to air for one hour at 28° C.

#### DISCUSSION

There is unanimity among the students of this subject that ascorbic acid in true serum of whole blood is significantly more stable than in separated serum. The presence of blood cells obviously protects serum ascorbic acid. From our experiments it now appears that the effects of red blood corpuscles and of leukocytes are essentially different. Ascorbic acid is completely protected in true serum containing erythrocytes only.

This stabilizing effect of red cells was also studied with varying numbers of cells. When calculated per one mgm. of ascorbic acid in serum, it appeared quite regularly that approximately 100,000 erythrocytes per cubic millimeter do protect such a concentration, while fewer cells do not.

Under the same conditions, *viz.* in air, leukocytes do not prevent, but rather enhance the deterioration of ascorbic acid in serum. Determina-

tions of ascorbic acid in whole blood carried out simultaneously proved that this decrease of serum ascorbic acid in air in the presence of leukocytes is due to actual loss and not to oxidation of ascorbic acid to its dehydro form. This effect of leukocytes obviously requires the presence of oxygen, since it did not occur under nitrogen. From these observations it would appear that erythrocytes and leukocytes affect oxidative and/or reducing processes in serum in essentially different ways. Neither of these influences seems to be due to changes in serum induced by cellular leakage; they seem rather to be dependent upon the presence of intact cells. In Figure 1C erythrocytes again had protected serum ascorbic acid, while erythrocytes plus leukocytes had promoted its deterioration up to the time indicated by arrows when both these sera were removed from their respective cells and further observed as separated sera. It then became evident that their rate of deterioration followed rather closely that of serum separated at the very beginning of the experiment.

Under these conditions of our experiments, ascorbic acid was transferred from serum to leukocytes but not to erythrocytes. This observation does not imply that erythrocytes do not contain ascorbic acid *in vivo*. It is quite possible that observations extended over longer periods of time would establish transfer of ascorbic acid from serum to erythrocytes, too. Such an extension of the duration of observation, however, involves the danger of cell volume changes or hemolysis which are prohibitive in studies of distribution. But even if transfer from serum to erythrocytes could be demonstrated *in vitro*, the amounts transferred could only be a fraction of those transferred to leukocytes.

A similarly significant difference between the ascorbic acid content of leukocytes and erythrocytes was described by Butler and Cushman (7) who noted a concentration of ascorbic acid in the former of about 40 times that in the latter. Crandon, Lund and Dill (8) confirmed this observation and further reported that leukocytes retain their ascorbic acid much longer than erythrocytes in the course of vitamin C deprivation. Our findings that *in vitro* transfer of ascorbic acid occurs chiefly from serum to leukocytes is in complete agreement with *in vivo* observations by Ralli

and Sherry (9). These authors noted decreased plasma concentrations of ascorbic acid following the administration of insulin. Upon further investigation, this decrease appeared to be due to a redistribution of serum ascorbic acid rather than to true loss. The decrease in plasma ascorbic acid, then, was found to be associated with a rise in the ascorbic acid content of leukocytes (and platelets), whereas no such increase could be demonstrated for red cells.

As in previously reported experiments (1, 2), transfers were studied at 37° C., with continuous agitation, in an atmosphere of nitrogen, the latter being essential for the prevention of oxidative destruction. The protective action of nitrogen was reestablished not only for separated sera but also for true serum separated after 45 and 90 minutes from blood containing only 1.4 million erythrocytes, but 6,800 leukocytes per cubic millimeter, respectively. As stated above, the latter type of cells caused increased destruction of serum ascorbic acid in air; since erythrocytes protected serum ascorbic acid even in air, transfer of ascorbic acid added to serum could also be studied in such an atmosphere with blood containing only erythrocytes. No transfer of ascorbic acid from serum to erythrocytes up to 5.0 million per cubic millimeter was detected in blood exposed to air.

This striking difference between erythrocytes and leukocytes in their reaction to large concentrations of serum ascorbic acid further supports earlier conclusions that the distribution of ascorbic acid in blood does not follow the laws of simple diffusion. It was demonstrated previously (1, 2) that the exchange of ascorbic acid between serum and cells (*a*) depends on the maintenance of physiological temperature, (*b*) is unidirectional, *viz.*, from serum to cells only and not vice versa; from the presently reported experiments it can be added that this exchange (*c*) involves only leukocytes. In other tissues, too, preferences similar to these observed among blood cells seem to prevail; the endocrine glands, *e.g.*, the adrenals, are recognized as containing significantly higher ascorbic acid concentrations than liver, kidney or muscle tissues.

Our observations that changes in protein content, subsequent to ultracentrifuging, did not affect the distribution of ascorbic acid in serum con-

firm findings by Coolidge (10) who mentions equal concentrations of ascorbic acid in substrate and ultrafiltrate of human serum obtained by his newly devised method.

The previously established irreversibility of the transfer of ascorbic acid from serum to cells (2) had been attributed to a combination of ascorbic acid with intracellular substances. In that connection, proteins had been mentioned because several communications referred to ascorbic acid as bound to this group. From our present studies, however, it is obvious that ascorbic acid is not bound to serum proteins. Nor did different concentrations or differences in composition of serum protein influence the stability of ascorbic acid when studied in an atmosphere of air.

#### SUMMARY

In an atmosphere of air, the deterioration of serum ascorbic acid is prevented by the presence of intact erythrocytes, while it is enhanced by that of leukocytes.

Under nitrogen, ascorbic acid added to serum is transferred to leukocytes but not to erythrocytes. Neither do the latter take up the vitamin in an atmosphere of air.

Ascorbic acid is not bound to serum proteins nor do different protein concentrations induce different rates of deterioration of serum ascorbic acid.

The influence of leukocytes was not studied separately but was estimated from studies of erythrocytes with and without leukocytes, the differences between the two being ascribed to leukocytes.

We are indebted to Miss M. Toothill who offered her most generous cooperation and performed the considerable number of blood counts involved in this investigation.

We wish to express our gratitude to Dr. K. G. Stern who kindly helped us in the use of the ultracentrifuge.

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# EVIDENCE FOR GENERAL DISTRIBUTION OF PERIPHERAL RESISTANCE IN COARCTATION OF THE AORTA: REPORT OF THREE CASES

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(Received for publication April 24, 1941)

The frequency with which elevation of arterial pressure attends stenosis (coarctation) of the arch of the aorta was first made clear several years ago by Lewis (1), and has recently been emphasized by King (2) in a complete review of cases in which estimations of arterial pressure have been made. King agrees essentially with Lewis when he says, "Hence, there is no well defined case so far recorded in which the circulatory compensation was above suspicion and in which the pressure in both arms was found to be normal." In this statement the upper limit of normal systolic pressure is taken to be 140 and diastolic, 100 mm. Hg.

Various explanations have been advanced to account for hypertension in the arms which occurs with such regularity in association with coarctation of the aorta. Blumgart, Lawrence and Ernestene (3) ascribed the hypertension to the resistance furnished by the constriction of the aorta and by the collateral passages. They held this view chiefly because arteriolar pressure in the upper extremities was found to be normal.

From studies of blood flow in the hand and upper arm, Prinzmetal and Wilson (4) and Pickering (5) came independently and almost simultaneously to conclusions which differed from the earlier notions of Blumgart and his co-workers. Both authors found that there was an increase in resistance to the flow in the upper extremities which was of general distribution, but they were unable to agree upon the mechanism by which the increase was brought about. Prinzmetal and Wilson believed that the increase in arteriolar tone was due to hyperactivity of the vasomotor nerves for, when the vasoconstrictor activity was inhibited by application of heat, the increase in the flow of blood through the hand was found to be much greater than in normal individuals or in individuals suffering from "essential" hypertension. The results of Pickering's studies were in accord with

Prinzmetal and Wilson so far as general distribution of increased resistance was concerned. Pickering was, however, unable to show any greater increase in flow of blood than that encountered in normal individuals either when heat was applied to the arm or when the nerves of the extremities had been injected with novocain. Evidence for the existence of nervous origin of the arteriolar hypertonus was lacking in his experiments. He pointed out that there were no known nervous pathways which could lead stimuli to affect only the vasomotor system in the upper half of the body and that the view that a substance circulating in the blood could give rise to contraction of the vessels in the upper half of the body only was likewise untenable. He suggested tentatively that some abnormality of the peripheral arterioles—such as hypoplasia of the glomi—might explain an increase in resistance in the upper extremities. This notion avoided the necessity of inferring an increase in tone confined to the arterioles of the upper half of the body.

Conclusions from these studies were drawn under the assumption that increased resistance was present *only* in the upper half of the body in coarctation of the aorta. When the facts upon which this assumption rests are more closely examined, it becomes evident that they are insufficient. First, the importance of diastolic pressure in drawing conclusions concerning peripheral resistance has not been recognized. A clear distinction between increase in *peripheral* or *arteriolar* resistance and increase in *elastic* resistance, due in coarctation of the aorta mainly to decrease in size of the arterial reservoir, needs to be made. For measuring peripheral resistance, diastolic, not systolic pressure is important. Second, measurements of pressure in the legs carried out by the auscultatory technique with pneumatic cuffs of a size suitable for arms, often in the presence of admittedly feeble Korotkow sounds, cannot be con-

sidered reliable enough to show with any degree of certainty that either level of arterial pressure is high, low or normal in the lower half of the body. Third, the number of satisfactory observations upon arterial pressure in the legs are too few.

The inadequacy of the information so far available made it desirable to describe direct simultaneous records of arterial pressure in radial and femoral arteries of three cases of coarctation of the aorta. The records were obtained by use of two Hamilton intra-arterial manometers. All three cases exhibited marked hypertension in the upper extremities and also many of the symptoms so frequently observed in instances of "essential" hypertension, such as emotional instability, headache and easily induced fatigue. Two of the cases were classified for several years in more than one clinic as "essential" hypertension.

*Case 1.*<sup>1</sup> *W. G.* A white male clerk, aged 30, was first seen at the hospital of the Rockefeller Institute on January 27th, 1936. He had always been nervous and moody and had been told at 10 years of age that he had high blood pressure. At 21 years of age he began to notice palpitation and mild precordial pain on effort and with excitement and headache. On examination the sounds of the heart were distinct, the rate regular but rapid and a loud systolic murmur was always present in the third right interspace. The walls of the radial arteries were readily palpable but elasticity as inferred from measurements of the velocity of the arterial pulse wave in the brachio-radial artery (6.4 meters per second) was within the range of normal. Brachial arterial pressure was very variable (170/96 to 230/130; on one occasion, 280/170). The pressure in the legs was not easily measured by auscultatory technique; it was, roughly, 120/100 with a pneumatic cuff 20 cm. wide. There were well-marked subscapular pulsations and murmurs. The femoral pulse was scarcely palpable. Renal function was normal, the basal metabolic rate varied between +6 and +15 per cent. Dr. Harold J. Stewart (Case 5, Stewart's (30) series) found his cardiac output to be 2.85 liters per square meter per minute, a moderate increase. At that time (September 1939) the basal metabolic rate was +25 per cent. X-ray photographs of the chest showed the notching of the ribs described by Railsbeck and Dock (6), and constriction of the aorta was visible. On fluoroscopic examination there was little or no pulsation below the constriction, while above it was very vigorous.

This patient is at present fairly well and working regularly as clerk-foreman. He complains still of fatigue, occasional palpitation and nervousness. His arterial pressure remains in the neighborhood of 220/120 mm. Hg.

<sup>1</sup> Cases 1 and 3 were kindly referred by Dr. William Goldring of New York University Medical School.

*Case 2. H. L.* A married woman, aged 46 at death on June 31st, 1939, came to this hospital November 14th, 1922 complaining of headache and dizziness. She stated that arterial hypertension had been discovered one year previously. She was followed at rather frequent intervals throughout the remainder of her life. Until the appearance of signs of heart failure in 1937, she was quite well. Renal function was always good. Pulsations were present over the lower part of the chest posteriorly. The femoral pulse was just palpable. Arterial pressures measured by the auscultatory technique in the brachial artery varied between 210 and 260 mm. Hg systolic, 120 and 180 diastolic; and in either leg between 100/96 and 120/100. The measurements in the legs were made with a wide cuff. X-ray photographs of the thorax exhibited the characteristic notching of the ribs from 1922 on. In May 1938 a right-sided hemiplegia occurred. She suffered an attack of lobar pneumonia in January 1939 and died six months later following what was apparently a cerebral vascular accident.

Postmortem showed a marked constriction of the aorta just above and at the level of the origin of the renal vessels. The lumen was reduced to about 2 mm. in diameter. The nature of the constriction is not yet clear.<sup>2</sup>

*Case 3. A. C.* A healthy youth, aged 16, was referred to this hospital for study on March 1st, 1939 because hypertension discovered a few months earlier on examination for working papers had lead to the diagnosis of coarctation of the aorta. His only complaints were that he became a little short of breath running with other boys and that he frequently had cramps in his legs at night after long walks (8- to 10-mile hikes).

He was a large-framed, healthy looking lad. The radial pulse was full and bounding. Pressure in either brachial artery varied between 170 and 200 mm. Hg systolic, 100 and 108 mm. Hg diastolic. The sounds of the heart were essentially normal. On both sides of the chest, between the lower part of the scapulae and spine, deep strong arterial pulsations were seen and felt. The femoral pulses were barely palpable; popliteal and pedal pulses were not felt. Measurements of arterial pressures in the leg with two sizes of cuff placed just above the knee were unsatisfactory. With a 20 cm. cuff, systolic pressure was estimated at 116, diastolic at 104; with a 12.5 cm. cuff, systolic and diastolic pressures were estimated at 120 and 108, respectively.

The circulation time was measured on two occasions by intravenous injections of a solution of magnesium sulphate and calcium gluconate (7). Fair agreement was obtained between the two measurements (Table I). It is of interest to note that on both occasions two distinct waves of heat were felt in the crotch, suggesting that part of the blood may have reached the perineum through the aorta and part through the collateral circulation.

The patient continues to attend school and leads an apparently normal life free of symptoms.

<sup>2</sup> This location of aortic constriction is distinctly rare. A detailed report of the case will appear elsewhere.

TABLE I

*Velocity of flow of blood (Case 3) measured by injection of a solution of calcium gluconate and magnesium sulphate on two occasions*

Test	Face Tongue	Chest	Arm	Hand	Crotch	Leg (Thigh)
1	12	14	18		20 and 31	24
2	12	17	21	24	22 and 29	26

Figures record in seconds the time of arrival of the sensation of heat at the various points indicated. Note the occurrence of sensation of heat twice in the perineal region (crotch).

### DISCUSSION

The valuable collection of material by King has made it a simple matter to bring up to date the facts known about the levels of arterial pressure in cases of coarctation. King collected 175 cases in which blood pressure measurements had been made and since that time forty-two have been reported, making a total of 217 (Table II) (8 to 26). As King points out, practically all exhibit elevation of *systolic* pressure in at least one arm (Table III). Since the state of peripheral resistance is the chief concern of the present discussion, it is important also to note that elevation of *diastolic* pressure (above 100 mm. Hg) in at least one arm was present in nearly half of the number of cases in which it was measured (Table III). Diastolic pressure in the legs was recorded in only sixty-five cases, and in about one-fifth (twelve cases), in spite of very small pulse pressures, it was above 100 mm. Hg. In four, it was higher in the legs than in the arms. It is plain, too, from frequent statements of the difficulty encountered in recording pressure in the legs by means of a pneumatic cuff and auscultation that these measurements may not be reliable. Even so, elevation of the diastolic pressure in the legs (and inferentially, increase in peripheral resistance) seems to be not altogether infrequent and the assumption that increase in peripheral resistance is confined to the upper half of the body does not appear to be warranted.

In the present study, direct simultaneous measurements of the levels of arterial pressure in radial and femoral arteries of the three cases of coarctation of the aorta just described were obtained by

the use of two intra-arterial manometers (27). Pressures recorded from the radial arteries of normal and hypertensive individuals in this manner differ but little from those recorded for the femoral, especially with regard to diastolic pressure. Systolic is usually slightly higher in the femoral. In indirect measurements, if a larger cuff is used for the legs (20 cm.) than for the arms (12 cm.), the agreement between these measurements and those obtained from the intra-arterial manometers is better than with a small cuff, but is still not satisfactory. The use of both methods (intra-arterial manometers and auscultatory technique with a large cuff) demonstrates that the diastolic pressure is roughly the same in the femoral as in the radial artery in both normal and hypertensive individuals.

The diastolic level of arterial pressure in the radial arteries was found, by direct measurement to be elevated in all three cases. The diastolic pressure in the femoral artery was above 100 mm. Hg in two cases (Figure 1, Case 1 and Figure 2, Case 3). In the case in which the diastolic pressure was below 100 mm. Hg in the legs (Figure 2, Case 2), not only a slight narrowing of the aorta in the region of the isthmus but almost complete occlusion just above the renal vessels was found on autopsy. This case is all the more interesting because of the great height of both levels of pressure in the arms. It was by clamping the aorta in this region, just above the renal arteries, that Page (28) was able to obtain the greatest elevation of pressure. The failure of the pressure to become elevated in the legs was probably due to the marked degree and the unusually low site of the constriction. Yet, with a pulse pressure of only 15 mm. Hg, the diastolic pressure in the legs remained above 90 mm. Pulse pressure and systolic pressure were, as can readily be seen in the tracings, much smaller in the femoral than in the radial artery in all three cases.

Study of a case of coarctation of the aorta by means of direct measurement of arterial pulse pressure was published by Woodbury, Murphey and Hamilton (29) while preparation of this manuscript was in progress. Their study includes a number of clear-cut detailed observations upon the dynamics of the circulation. It is important to note that these authors find only small differences between diastolic pressure in femoral and



TABLE II

*Cases of coarctation of the aorta reported in the literature since 1937*

Number	Author	Sex	Age	Blood pressure		Color	Autopsy
				Arm	Leg		
1	Appelbaum, E., and Kalkstein, M.	M.	38	R. 180/130 L. 240/114	Oscillometer reading 150/100	W.	No
2	Barsantini, J. C., and Bazzano, J. J.	M.	24	R. 165/100 L. 170/100		W.	Yes
3	Benkowitz, K. B., and Hunter, W. C.	M.	67	R. 180/85 L. 180/80		?	Yes
4	Gitlow, S.	M.	26	152/90 160/100	123 by palpation	W.	No
5	Goodson, W. H.	M.	16½	$\frac{150}{90}$ (average)	$\frac{110}{90}$	W.	Yes
6	Goodson, W. H.	F.	19	R. 164/92 L. 152/80	Could not be read	W.	No
7	Hills, R. G.	M.	16	R. 180/100 L. 110/85	110/90	W.	No
8	Klemola, E.	M.	27	220/110	?	W.	No
9	Klemola, E.	M.	20	R. $\frac{160-165}{80}$ L. $\frac{145-155}{80}$	R. $\frac{125-135}{80}$ L. 135/80	W.	No
10	Klemola, E.	M.	19	200/100	?	W.	No
11	Klemola, E.	?	22	140/90		W.	No
12	McNair, J. S.	M.	11	154/?	90/70	?	No
13	Parker, R. L., and Dry, T. J.	M.	26	R. 210/40 L. 100/78	No pulse felt	?	Yes
14	Regester, R. P., and Innes, M. B.	M.	19	R. 190/150 to 190/124 L. 130/90	Not taken	W.	Yes
15	Ernstene, A. C., and Robins, S. A.	M.	47	164/94	86/76	W.	No
16	Rooke, E. J.	M.	3	R. 150/85 L. 110/? 1 month later 162/?	Not taken	W.	No
17	Sheldon, W.	M.	12	150/100	No pulse	W.	No
18	Steele, J. M.	M.	29	R. 170/98 R. 230/130	R. 120/100	W.	No
19	Steele, J. M.	F.	45	R. 170/120 R. 282/168	R. 100/94 120/98	W.	Yes
20	Steele, J. M.	M.	16	R. 170/96 R. 200/108	R. 120/108	W.	No
21	Venzoni, M.	M.	24	200/120		W.	Yes
22	Walker, J. B., and Livingstone, F. D. M.	M.	17	178/100 215/110	110/90	W.	Yes

TABLE II—Continued

Number	Author	Sex	Age	Blood pressure		Color	Autopsy
				Arm	Leg		
23	Wechsler, H. F., and Gustafson, E.	M.	30	R. 160/50 L. 190/56	R. 180/80 L. 104/80	W.	Yes
24	Weinberg, T. B., and Gartenlaub, C.	M.	23	R. 150/90 to 170/90 L. 100/90	Not obtainable	W.	No
25	Weinberg, T. B., and Gartenlaub, C.	F.	41	R. 200/76 L. 194/72	R. 94/80 L. 92/76	?	?
26	Wilkinson, K. D.	F.	5	150/80	Feeble pulse	W.	No
27	Wilkinson, K. D.	M.	14	198/135	No pulse	W.	No
28	Wolke, K.	F.	38	210/105 210/110	Unsatisfactory	W.	No
29	Wolke, K.	F.	36	R. 160/100 L. 160/100	Unsatisfactory	W.	No
30	Woodbury, R. A., Murnphey, E. E., and Hamilton, W. F.	M.	26	164/100	130/100	W.	No
31	Stewart, H. J., and Bailey, R. L.	?	15	R. 170/90 L. 160/87	R. 100/80 L. 95/90	W.	No
32	Stewart, H. J., and Bailey, R. L.	?	28	R. 136/70 L. 134/72	R. 120/90 L. 115/90	W.	No
33	Stewart, H. J., and Bailey, R. L.	?	23	R. 174/60 L. 134/72	R. 98/82 L. 96/84	W.	Yes
34	Stewart, H. J., and Bailey, R. L.	?	9	R. 200/120 L. 195/120	R. 125/105 L. 150/145	W.	No
35	Stewart, H. J., and Bailey, R. L.	?	38	R. 165/110 L. 180/110	R. Not L. obtainable	W.	No
36	Stewart, H. J., and Bailey, R. L.	?	64	R. 200/100 L. 190/90	R. Not L. obtainable	W.	Yes
37	Stewart, H. J., and Bailey, R. L.	?	22	R. 160/86 L. 160/80	R. 110/80 L. 108/80	W.	No
38	Stewart, H. J., and Bailey, R. L.	?	27	R. 200/100 L. 230/130	R. 125/110 L. 125/110	W.	No
*39	Stewart, H. J., and Bailey, R. L.	?	48	R. 255/160 L. 240/160	L. 290/200	W.	Yes
40	Stewart, H. J., and Bailey, R. L.	?	16	R. 168/80 L. 138/80	R. Not L. obtainable	W.	Yes
41	Stewart, H. J., and Bailey, R. L.	?	24	R. 220/120 L. 180/120	R. 120/98 L. 108/100	W.	Yes
42	Stewart, H. J., and Bailey, R. L.	?	26	R. 170/70 L. 170/70	R. Not L. obtainable	W.	No

Cases are listed in a manner comparable to that employed in King's paper.  
 \* This patient did not have coarctation sufficient to produce any obvious alterations in the hemodynamics of the circulation.

of the

TABLE III

Frequency of elevation of levels of arterial pressure in cases of coarctation of aorta

	Total number cases	Number elevated*	Per cent elevated
Cases with measurements of <i>systolic</i> pressure			
In at least one arm.....	216†	195	90.
In at least one leg.....	98	10	10.
Cases with measurements of <i>diastolic</i> pressure			
In at least one arm.....	194	86	44.2
In at least one leg.....	73	16	22.

\* Pressures above 140 mm. Hg systolic and 100 mm. Hg diastolic were considered elevated.

† One case (Number 39, Table II) was omitted since the degree of coarctation was so slight as to be insignificant in disturbing hemodynamic relationships and also because well-marked renal lesions were present at autopsy.

radial arteries—88 in the arm and 82 in the leg. These values for diastolic pressure are not indicative of an increase in peripheral resistance either above or below the coarctation but their close agreement suggests that peripheral resistance above the coarctation does not differ from that encountered below it. On several occasions, however, high values for diastolic pressures, both above and below the coarctation, were recorded during control periods (radial artery 111, dorsalis

pedis 98 mm. Hg; radial artery 121, dorsalis pedis 110 mm. Hg). Such high levels are rarely encountered in normal persons and suggest that unusual increases in peripheral resistance were not infrequent in this individual.

It is plain that in coarctation of the aorta, in spite of a very low systolic pressure in the legs, a level of diastolic pressure comparable to that

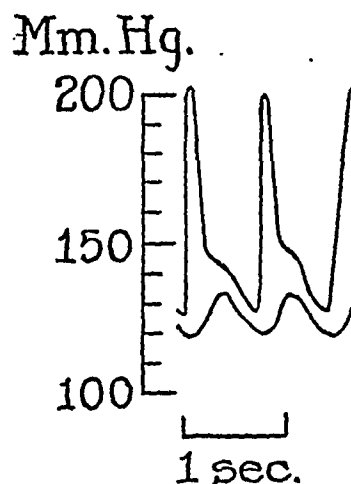


FIG. 1. SIMULTANEOUS TRACINGS OF ARTERIAL PRESSURE FROM THE FEMORAL AND RADIAL ARTERIES (CASE 1) REDRAWN TO THE SAME SCALE ILLUSTRATE THE DIFFERENCE IN PULSE PRESSURE

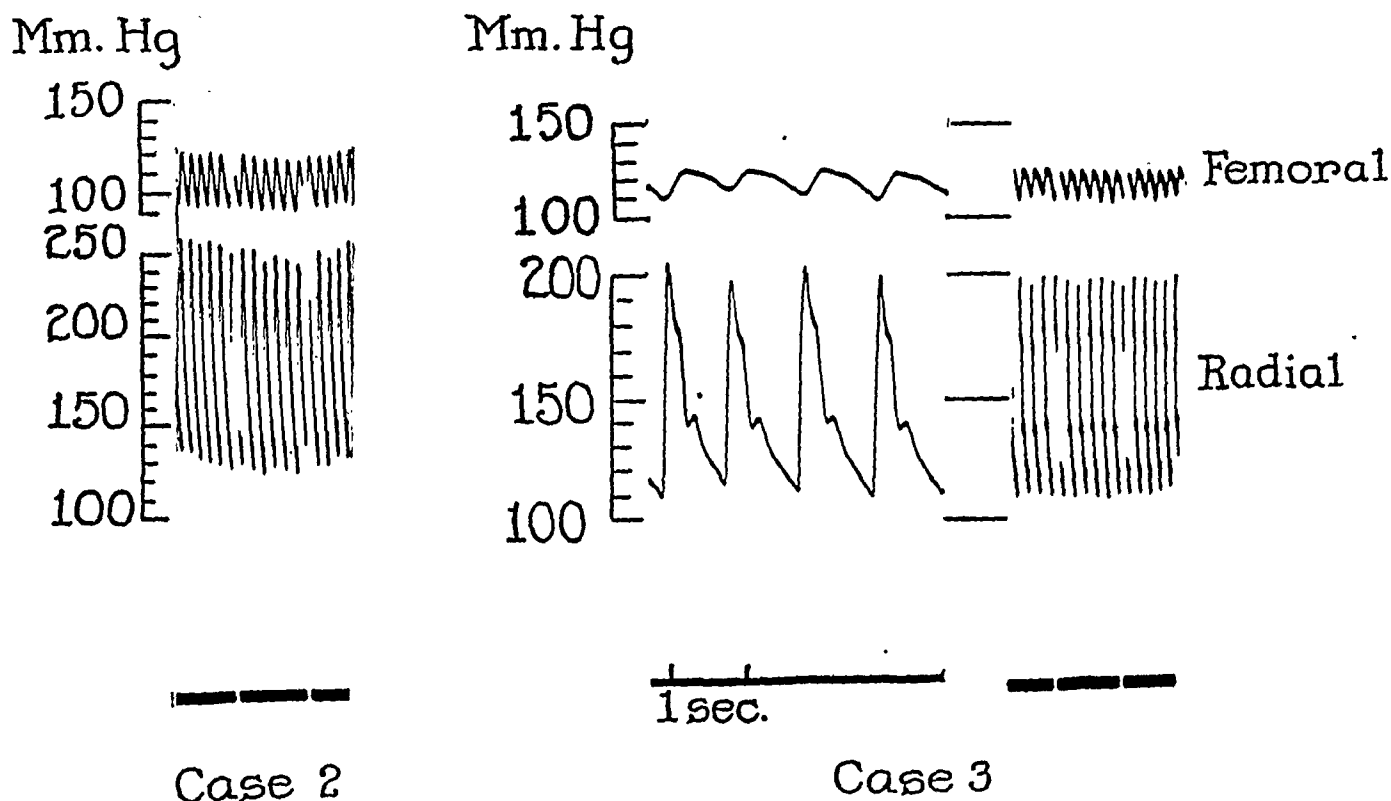


FIG. 2. SIMULTANEOUS PHOTOGRAPHIC RECORDS OF ARTERIAL PRESSURE IN THE RADIAL AND FEMORAL ARTERIES OF TWO CASES OF COARCTATION OF THE AORTA (CASES 2 AND 3) ARE REPRODUCED

in the arms often exists. Increase in cardiac output does not ordinarily account for the great increase in pressure which is frequently observed (30), although cardiac output may sometimes be large enough, as in one of Stewart's cases (30), to account for mild rises in arterial pressure. These observations, together with the knowledge that the flow of blood in the legs is no greater than normal (1, 3), strongly suggest that peripheral arteriolar tone is increased throughout the body just as it is in "essential" hypertension.

Evidence has been obtained that, following constriction of the aorta in animals, hypertension in the upper half of the body occurs *only* when the constriction is above the orifices of renal vessels (31, 32, 33). The inference is, of course, that hypertension is due to interference with the renal blood supply as is the case when the renal arteries themselves are constricted. It has also been shown in dogs that increase in tone of the peripheral arterioles takes place under these circumstances in the lower as well as in the upper extremities (34). Although the pulse pressure is very small in the femoral arteries of dogs whose aortas have been constricted above the renal vessels, diastolic pressure may be higher than it was before the aorta was constricted.

Constriction of the renal arteries in dogs, rabbits and monkeys regularly gives rise to increase in diastolic pressure throughout the body. It now appears that in dogs, in the same way, constriction of the aorta above, but not below the orifice of the renal vessels, also gives rise to an increase in diastolic pressure both in the upper and lower extremities. The present observations in patients show that the increase in diastolic pressure often occurs in the legs as well as in the arms in coarctation of the aorta. Elevation of diastolic arterial pressure does not then depend upon some local or mechanical disturbance, but upon a systemic reaction of the arterioles and in this respect resembles that which follows constriction of the aorta above the renal vessels in dogs and constriction of the renal arteries themselves.

Evidence not entirely in accord with this interpretation of the facts exists. For one thing, individuals suffering from coarctation of the aorta rarely, if ever, develop renal insufficiency in the common sense of the term. Their death is commonly due to heart failure or apoplexy and not to

renal failure. The recent clear demonstration that renal blood flow is markedly reduced in patients with coarctation of the aorta furnishes grounds for the belief that associated arterial hypertension is renal in origin (35). There are also the studies of Levy and Blalock (36) and, more recently, those of Brothner (37) suggesting that the proportion of the total blood flow intercepted by constriction of the aorta is important in the development of hypertension. Brothner's study was, however, carried out only within a period of 40 minutes after constriction and may have little relation to subsequent events. This temporary elevation of pressure was shown in 1931 by Barcroft (38) to be practically abolished by shutting off the return flow from the inferior vena cava and presumably had to do with redistribution of blood. It is possible, also, that elevation of diastolic pressure in the lower extremities may depend upon the belated delivery of blood to this region because of its circuitous route. The degree of delay (roughly 0.05 to 0.07 second) of the maximal pressure, as measured in Case 2 and 3, would hardly seem to account for the observed increase in minimal pressure.

#### SUMMARY AND CONCLUSIONS

1. Review of the knowledge of levels of arterial pressure in 217 cases of coarctation of the aorta makes untenable the assumption that increase in peripheral resistance is situated in the upper half of the body only.
2. In two of three cases of coarctation of the aorta, diastolic pressure has been shown by intra-arterial measurement to be elevated above 100 mm. Hg in the femoral, as well as in the radial arteries. This is interpreted as evidence of general increase in arteriolar tone throughout the body.
3. In so far as the distribution of peripheral arteriolar resistance is concerned, arterial hypertension in coarctation of the aorta does not differ from the common forms of arterial hypertension.

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# TREATMENT OF CIRRHOSIS OF THE LIVER BY A NUTRITIOUS DIET AND SUPPLEMENTS RICH IN VITAMIN B COMPLEX

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The present paper reports observations on 54 patients with cirrhosis of the liver who were treated by means of a highly nutritious diet together with vitamin B concentrates. The basis for this treatment rests on several clinical and experimental observations. It is known that the incidence of cirrhosis of the liver is high in certain countries where nutritional deficiencies are endemic (1, 2). Although malaria is blamed popularly for the high incidence of cirrhosis in these countries (3, 4), there is no direct evidence that malaria produces cirrhosis of the liver (5, 6). The association of cirrhosis and malaria suggests that the latter is a predisposing factor rather than a primary etiologic agent. The coincidence of enteric diseases with cirrhosis of the liver and with deficiency diseases may be explained similarly. In the western hemisphere cirrhosis of the liver, like beriberi and pellagra, is often associated with chronic alcoholism. Since "alcoholic" beriberi (7) and "alcoholic" pellagra (8) have been shown to be similar to the endemic beriberi and pellagra both in symptoms and response to therapy, it has been concluded that alcoholism merely predisposes to these nutritional deficiency diseases. It seemed possible that the correlation between alcoholism and cirrhosis of the liver might also be due to coexisting nutritional deficiency.

Experimental evidence to support this hypothesis has accumulated in recent years. It has been known that starvation renders the liver more vulnerable to injury by hepatotoxins (9). Lack of certain food factors contained in yeast are said to cause fatty changes in the liver (10) and impaired function (11). Recent studies by György (12) and by Rich (13) and their coworkers reveal that cirrhotic changes may possibly be produced in the rat and rabbit by feeding a diet deficient in unknown factors contained in yeast. It has also been shown that the feeding of excess fat (14) or of

excess cystine (15) results in fibrotic changes in the liver. Other studies indicate a protective action against hepatotoxins by the feeding of yeast (16, 17) or of high protein diets (18, 19, 20). It is possible that a balance of food factors may be essential to the integrity of a complex organ like the liver.

In a preliminary report on the treatment of 13 patients with alcoholic cirrhosis of the liver (21), it was noted that, in addition to signs of liver failure, there was evidence of specific malnutrition, notably of the vitamin B complex. These patients were fed a nutritious diet together with vitamin supplements. The improvement that followed treatment appeared to be outside chance expectations. However, since there is much variability in the degree of liver failure of different patients, and since these patients were observed for only one year, it seemed advisable to extend the program over a longer period of time. It was also important to compare their course with that of a similar group of hospitalized patients who had not received special dietary therapy.

## CASE MATERIAL

The patients were obtained by transfer from municipal hospitals in New York City, and from the wards of private hospitals. Therefore, as an economic group they are composed of the underprivileged class. They may be considered "unselected" in regard to the stage of their liver disease.<sup>1</sup> Since they were hospitalized because of liver failure, they represent a more advanced degree of liver incompetence than patients who are ambulatory, or who are being treated in outpatient clinics.

<sup>1</sup> Excluded from this series are 2 patients who underwent splenectomy and omentopexy 7 and 6 years, respectively, before entering the Research Service.

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## CASE MATERIAL

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TABLE I

*Clinical data on patients with cirrhosis of the liver*

	Treated series (54 patients)					Control series (386 patients)	
	Group 1 20 patients recovered	Group 2 12 patients improved	Group 3 22 patients failed	Total number	Total per cent	Number	Per cent
Average age, years.....	48	51	52			50	
Sex, males.....	10	9	16	35	65	267	69
females.....	10	3	6	19	35	119	31
Antecedent factors							
Syphilis.....	4	5	5	14	26	62	16
Arsphenamine.....	3	3	5	11	20	34	9
Alcoholism.....	18	11	18	47	87	207	54
Malnutrition.....	17	9	18	44	81	67	17
Symptoms							
Weight loss.....	16	9	17	42	78	206	53
Abdominal swelling.....	15	12	21	48	89	301	80
Peripheral edema.....	16	9	21	46	85	236	61
Nausea and vomiting.....	16	6	7	29	54	129	33
Hematemesis.....	7	2	4	13	24	106	27
Abdominal pain.....	8	4	10	22	40	121	31
Signs							
Ascites.....	15	12	21	48	89	301	80
Palpable liver.....	20	11	13	44	81	291	75
Palpable spleen.....	15	10	13	38	70	170	44
Jaundice.....	14	8	12	34	63	252	65
Edema.....	15	9	22	46	85	236	61
Hemorrhoids.....	9	6	15	30	56	105	27
Collateral veins.....	11	11	19	41	76	91	23
Vascular spider.....	14	10	13	37	68	58	15
Atrophy of tongue.....	15	7	10	32	60		
Dermatitis.....	12	5	17	34	63		
Polyneuritis.....	12	2	7	21	40	40	13
Bromsulfalein dye retention.....	18	12	22	52	96		
Positive Takata-Ara test.....	16	12	21	49	91		
Average serum albumin.....	3.1	2.8	2.4	average 2.7			
Average serum globulin.....	3.7	4.1	3.9	average 3.9			

The bromsulfalein dye test was done by the method of Rosenthal and White (a), using 5 mgm. of dye per kilo body weight. The tabulated figures refer to the per cent of dye retained by the blood serum after 30 minutes. The Takata-Ara test was done by the method of Heath and King (b). The serum proteins were determined by the Howe method (c).

(a) Rosenthal, S. M., and White, E. D., J. A. M. A., 1925, 54, 112.

(b) Heath, C. W., and King, E. F., New England J. Med., 1934, 211, 1077.

(c) Howe, P. E., J. Biol. Chem., 1921, 49, 109.

#### *Clinical data—comparison with a control series*

A tabulation of certain antecedent factors, symptoms, and signs is made in Table I for the 54 patients in this study. The patients are divided into three groups in order to show (1) those patients who made clinical recovery, (2) those patients whose disease was arrested or who showed partial improvement, (3) those patients who died of progressive liver failure. In the right half of the table are listed similar data obtained from a control group of hospitalized patients ill of cirrhosis of the liver. The control data are part of a statistical study of 386 cases obtained from the rec-

ords of five New York hospitals during the past 20 years (22).

Several differences are apparent between the two series. The incidence of syphilis was higher in the treated series (26 per cent) than in the control series (16 per cent). Accordingly, a larger number of patients in the treated series was exposed to arsenicals. In both groups the diagnosis of syphilis was considered positive if there was a history of the treated disease, if there were clinical signs of syphilis or if repeated serological tests were positive. The possible etiologic significance of syphilis and of arsphenamine therapy in portal

cirrhosis has been discussed by others (23, 24, 25). Although 14 patients in the treated series had syphilis, none showed histological evidence of syphilitic involvement of the liver.

In the treated series of patients the higher incidences of alcoholism, malnutrition, and weight loss in the pre-hospital period probably are due to more complete histories. Evidence of malnutrition was striking. It may be seen in the table that 81 per cent had eaten poor diets, and that 78 per cent had lost weight (allowing for ascites). Although careful dietary histories were taken, the data were not reliable enough to measure quantitatively. The most constant defects in the diets lay in the sparing use of *meat* and *dairy* products. Many patients gave histories of having subsisted entirely on alcoholic liquor for intervals of several days and of having refused all food during these sprees.

The specific deficiency of the vitamin B complex is shown in the treated series by the high incidence of polyneuritis (40 per cent), of "pellagrous" dermatitis (63 per cent) and of atrophy of the tongue papillae (60 per cent).

The diagnosis of polyneuritis was based on signs of bilateral sensory changes (hypo- or hyperesthesia), loss of vibratory sensation, and loss of tendon reflexes in the lower extremities. Twenty-one patients showed these signs. In addition, 3 had the above changes without loss of tendon reflexes; 6 others showed the loss of vibratory sensation alone. The inclusion of these probable cases would increase the incidence of polyneuritis in the series from 40 to 57 per cent.

The relative incidence and severity of polyneuritis were greater in the females than in the males. The association of polyneuritis with cirrhosis of the liver and this peculiar sex difference have been noted by Wayburn and Guerard (26) and others. The "pellagrous" dermatitis refers to the presence of dry, scaly skin which at times was generalized and at times was limited to the extremities. Although these changes were definite, they were not associated with the inflammatory edema, severe erythema, and diarrhea that characterize frank pellagra. Except for 4 or 5 instances with marked glossitis, the atrophy of the tongue papillae was painless and of moderate degree.

In the control series of patients there are few recorded instances of vitamin deficiency, although 13 per cent showed signs of polyneuritis. It is probable that these deficiencies were overlooked, since they were not popularly known until recent years, and since the control data cover a span of 20 years.

Data showing the symptoms and signs of liver failure reveal a close agreement between the

treated series and the control series of patients. It is assumed, therefore, that the severity of the underlying disease process is comparable in the two series of patients.

In the treated series certain other signs have been observed that have not been emphasized generally in the literature. Low-grade fever unrelated to infection and lasting for weeks or months has been noted in one-half the patients. Mental changes unrelated to recent alcohol intake were seen in one-third of the patients. In most instances, these were characterized by confusion and euphoria. In others a torpor was seen that simulated late cholemia. Lateral nystagmus was seen in 10 patients of whom 9 were women. These nervous changes, like polyneuritis, may be the result of coexisting nutritional deficiency (27, 28).

Recurrent nosebleeds were noted in 25 (46 per cent) of the cases. Vascular "spiders" of the skin (30) were observed in 68 per cent of the cases. Macrocytic anemia, usually moderate in degree, was present in 40 per cent of the patients. Except in 2 patients with hypertensive heart disease and 2 others who may have had beriberi heart, there was no evidence of heart failure in these patients. In 20 patients with ascites the venous pressure in the antecubital vein and the circulation time were within normal limits.

Certain laboratory data from the treated series are likewise included in the table. In 96 per cent the blood serum showed abnormal retention of bromsulfalein dye; in 91 per cent, a positive Takata-Ara test. The initial serum albumin value was low (below 4.0 grams per cent) in 96 per cent of the patients. The initial serum globulin value was high (above 3.0 grams per cent) in 83 per cent of the patients. It is noteworthy that the average initial value for serum albumin is higher in those patients who made clinical improvement than in those who failed to improve.

#### *Program of therapy*

In Table II is outlined the dietary regimen employed. The diet differs radically from that commonly advocated in this country for the treatment of cirrhosis of the liver, which is high in carbohydrate but low in protein and fat. The present diet contains a moderate amount of protein and fat. In addition to the protein of the diet (114 grams) the patients receive 50 grams of powder,

TABLE II  
Standard diets for cirrhosis patients

Semi-liquid diet						Solid diet					
			Proteins	Fats	Car-bohy-drates				Proteins	Fats	Car-bohy-drates
7 a.m.	Milk	200 cc.	6	8	10	<i>Breakfast</i>					
8 a.m.	Cereal (Pabulum)	100 grams	2	1	11	Fruit 18%	1 serving				18
	Sugar	12 grams			12	Cooked cereal or	200 grams	4	2		20
	20% Cream	30 cc.	1	6	1	Prepared cereal	30 grams				
	Eggs	2	13	10		Sugar on cereal	12 grams				12
9 a.m.	Orange juice	200 cc.			18	Eggs	2 only	13	10		
10 a.m.	Eggnog					Milk	200 cc.	6	8		10
	Milk	150 cc.	4.5	6	7.5	Toast	60 grams	4	1		30
	Egg	1	6.5	6		Butter	20 grams		17		
	Sugar	10 grams			10	Coffee					
	Brewer's yeast	25 grams	12.5	0.5	8.5	Cream 20%	30 cc.	1	6		1
						Sugar	12 grams				12
11 a.m.	Cream soup	200 cc.	6	14	15	9 a.m. Brewer's yeast	25 grams	12.5	0.5		8.5
	Mashed potatoes	100 grams	2	6	15	Milk	150 cc.	4.5	6		7.5
	Butter	10 grams		8.5		Sugar	12 grams				12
	Purée vegetables	100 grams			9	<i>Dinner</i>					
	Orange juice	200 cc.			18	Meat, medium fat	100 grams	17	20		
12 noon	Cocomalt	200 cc.	6	10	20	Vegetables 5%	100 grams	1			4
2 p.m.	Eggnog	200 cc.	11	11	17.5	Vegetables 10%	100 grams	2			9
3 p.m.	Orange juice	200 cc.			18	Vegetables 20%	100 grams	3			19
						Bread	30 grams	2			15
						Butter	20 grams		17		
4 p.m.	Cereal (Pabulum)	100 grams	2	1	11	Dessert (Cake, pudding)	1 serving	5	8		25
	Sugar	12 grams			12	Milk	200 cc.	6	8		10
	Cream 20%	30 cc.	1	6	1	Coffee					
	Jello	100 grams	1		18	Cream 20%	30 cc.	1	6		1
	Cream 20%	30 cc.	1	6	1	Sugar	12 grams				12
	Orange juice	200 cc.			18						
5 p.m.	Cocomalt	200 cc.	6	10	20	2 p.m. Brewer's yeast	25 grams	12.5	0.5		8.5
6 p.m.	Eggnog	200 cc.	11	11	17.5	Milk	150 cc.	4.5	6		7.5
	Brewer's yeast	25 grams	12.5	0.5	8.5	Sugar	12 grams				12
7 p.m.	Eggnog	200 cc.	11	11	17.5	3 p.m. orange juice	200 cc.				18
						<i>Supper</i>					
						Soup (Julienne)	200 cc.	4			4
						Meat, medium fat	100 grams	17	20		
						Vegetables 5% salad	100 grams	1			4
						Vegetables 20%	100 grams	3			19
						Bread	30 grams	2			15
						Butter	20 grams		17		
						Milk	200 cc.	6	8		10
						Fruit 18%	100 grams				18
						Tea					
						Cream 20%	30 cc.	1	6		1
						Sugar	12 grams				12
						7 p.m. Milk	200 cc.	6	8		10
Grand total			116	131	315	Grand total			139	175	365
Calories 2903						Calories 3591					

The semi-liquid diet was used only in the exceptional cases when patients either refused or were unable to take the solid diet. Brewer's yeast was fed in a milknog, according to the method described by Spies, Chinn, and McLester (a). The yeast was stirred thoroughly in cold milk, and flavored with sugar, vanilla, and nutmeg.

The writers are indebted to Miss Josephine Henneberger for the preparation of the diets.

(a) Spies, T. D., Chinn, A. B., and McLester, J. B., J. A. M. A., 1937, 108, 853.

Brewer's yeast<sup>2</sup> daily, of which the protein content is about 50 per cent. There has been no evidence of intolerance to fat in the amounts given. The stools do not contain excessive fat. Salt intake is restricted in patients with ascites and edema to the extent of omitting a salt shaker from the tray, and fluids are allowed up to 2,000 cc. daily. In the first group of 13 patients reported (21), vitamin B "complex" was provided daily in the form of autolyzed yeast (Vegex) or

of aqueous liver extract (Valentine). Brewer's yeast was then substituted for these concentrates because it was found to be more palatable in large amounts. In addition to the yeast, the patients generally received intramuscular injections of concentrated liver extract, 5 cc. twice weekly (Lilly or Lederle), and of thiamin chloride,<sup>3</sup> 5 mgm. daily. In patients with severe neuritis, thiamin chloride was administered in doses of 10 to 20

<sup>2</sup> Harris Laboratories, Tuckahoe, N. Y.; Mead Johnson & Co., Evansville, Indiana.

<sup>3</sup> Thiamin chloride was generously supplied by Merck & Co., Rahway, N. J., and by the Winthrop Chemical Co., New York City.

mgm. daily. Concentrates of vitamins A and D were fed only to the first series of 13 patients (21).

#### *Grouping of patients according to clinical course*

For the sake of convenience, the patients were placed in three groups, as described previously (Table I). The case histories of the patients are listed according to these three groups. Group 1 includes those whose course was characterized by steady improvement with clinical recovery; Group 2 includes those who made partial clinical improvement and also those cases who improved but who were followed too short a time to permit inclusion in Group 1; Group 3 includes those who died after progressive liver failure.

The typical patient with cirrhosis of the liver might be described as follows: Characteristically, he has been bedridden for weeks or months. He has lost much weight. At entry he is weak and tired, mentally dull and at times confused. Food is loathsome. He complains of thirst, of abdominal fullness and pain. There is often low-grade fever. The pulse rate is rapid. Breathing is shallow because of an elevated diaphragm. The urine output varies from 300 to 1,000 cc. daily. Abdominal hernia, hemorrhoids, nosebleeds, and polyneuritis may contribute to his disability. In those patients who fail to respond to treatment, the above symptoms and signs persist and become more severe. Ascites accumulates rapidly. Jaundice may become intense, and generally, after 1 to 3 months in the hospital, the patient dies in cholemia from intercurrent infection or from hemorrhage.

The changes that indicate improvement are gradual and slow in appearing. The nursing care at this critical stage must be as meticulous and vigilant as that given the patient with typhoid fever. Subsidence of fever, recovery of appetite, return of mental clarity and gain in strength gradually take place. With increase in the urinary output the ascites and edema disappear. This transition usually requires about 2 months. It has varied from 2 weeks to 10 months.

Although jaundice usually is of low grade, it takes weeks or months for this to subside completely. In most cases no appreciable change is noted in the size of liver or spleen. However,

abdominal pain and tenderness always subside with the patient's clinical improvement.

Six patients who had hematemeses in the past have had no recurrence for from 2 to 4 years. The cessation of nosebleeds has been observed in many patients; the disappearance of vascular "spiders" has been observed in 10 patients; and the reappearance of menses after protracted amenorrhea has been experienced by 4 women.

Changes in the laboratory findings have accompanied the clinical improvement. In 33 cases the value for serum albumin has increased significantly. The elevated serum globulin has fallen in 23 cases. The Takata-Ara test has changed from positive to negative in 19 instances. A diminished retention of bromsulfalein dye has accompanied improvement in 18 instances. The degree of change in the dye test, however, has not been striking. Changes in the cephalin flocculation test have reflected improvement in a significant number of cases. These findings will be reported elsewhere by Hanger (32). In almost all cases the blood counts and hemoglobin approached normal values.

Recovery from polyneuritis in these patients is slow and usually incomplete. Functional improvement is striking in most instances, but the return of vibratory sensation may be partial, and the tendon reflexes may fail to return even after several years of intensive treatment with vitamin B concentrates. The pellagrous dermatitis and the atrophy of the tongue papillae generally respond to therapy within a few weeks.

*Group 1. Patients whose course showed clinical recovery from symptoms and signs of cirrhosis.* It may be seen in Table I that this group of 20 patients with few exceptions showed plain evidence of liver decompensation before treatment was begun. Improvement was gauged by the following criteria: (1) gain in weight and strength, permitting the patient to resume fully his previous activities; (2) loss of ascites, edema, and jaundice without recurrence; (3) changes of serum proteins, Takata-Ara and bromsulfalein dye tests towards normal values.

Included in this group are 5 patients (Cases 22, 23, 24, 25, 30) who lost ascites and who were maintained in good health, but who died from complications that were not ascribable to liver failure.

*Group 2. Patients who made partial improvement.* There are 12 patients whose course showed signs of partial improvement. Five of these correspond in the degree of improvement to that noted in Group 1. Because the follow-up after discharge from the hospital has been limited to 4, 5, 6, 6, and 8 months, respectively, they are classed tentatively in Group 2. Three other patients have remained ascites-free for many months after having required abdominal taps in the past, but they are not in robust health. Although the serum albumin increased, it did not reach normal values in this group. Finally, there are 4 patients whose ascites disappeared and who were ambulatory but not vigorous. They subsequently died after being ascites-free for more than 2 years in each instance (cf Cases 26, 27, 28, 29).

*Group 3. Patients who died after progressive liver failure.* The survival period of this group of 22 patients varied from 2 days to 14 months after entry to the hospital. Only 4 of this group survived more than 5 months after entry. It is noteworthy that 11 of the 22 deaths occurred within 1 month of admission to the hospital. In many instances they were able to eat but a small portion of the prescribed diet. Histories of Cases 11, 19 and 21 are included since they are unusual. Histories of Cases 8 and 13 are included as typical of the remaining case histories in this group.

*Effect of administration of alcohol.* Since the routine treatment of these patients involves not only dietetic care but also the denial of alcoholic liquors, it seemed pertinent to test the effect of the administration of alcohol on a selected group. These 4 patients (Cases 41, 51, 46, 44) previously had strong alcoholic backgrounds. On admission to the hospital they presented varied degrees of liver failure. After they had shown signs of clinical improvement they were fed alcohol in addition to the nutritious diet and vitamin B concentrates described. Nine ounces of 40 per cent alcohol were fed daily as a fruit juice tonic for 6, 6, 14 and 18 months, respectively. There was no recrudescence of their former symptoms or signs, such as jaundice or ascites. The bromsulphalein dye test, Takata-Ara test, and the serum proteins showed no adverse changes. A fifth patient (Case 42), a barkeeper who has resumed his former trade, admits drinking six to eight glasses of beer daily in the past 1½ years since discharge from

the hospital. There has been no relapse in the clinical status of this patient who was severely decompensated at entry.

*Causes of death.* The causes of death in the fatal cases are listed in Table III. There are 14

TABLE III  
*Causes of death in 31 patients with cirrhosis of the liver*

	Number	Autopsies
Cirrhosis of the liver* . . . . .	14	8
Cirrhosis of the liver and massive hematemesis . . . . .	3	3
Cirrhosis of the liver and intra-abdominal hemorrhage . . . . .	3	2
Cirrhosis of the liver and Pick's disease . . . . .	1	1
Cirrhosis of the liver and peritonitis (purulent) . . . . .	1	1
Cirrhosis of the liver and pericarditis (purulent) . . . . .	1	1
Cirrhosis of the liver and portal thrombosis . . . . .	2	1
Cirrhosis of the liver and neoplasm (?) . . . . .	1	0
Cirrhosis of the liver and embolus (?) . . . . .	1	0
Gunshot . . . . .	1	1
Primary carcinoma of liver and portal thrombosis . . . . .	1	1
Pyelonephritis—uremia . . . . .	1	1
Cerebral thrombosis . . . . .	1	1
Total . . . . .	31	21

\* Terminal lobular pneumonia in 7 cases.

cases in which cirrhosis of the liver was the apparent primary cause of death. In addition to these are listed 13 instances in which certain complications of cirrhosis precipitated death. Finally, there are listed 4 cases in which the cause of death was unrelated to cirrhosis of the liver, although histological evidence of cirrhosis was present as well.

*Statistical comparison of the present (treated) series and a control series with respect to the duration of life*

In order to estimate the effectiveness of the dietary regimen employed here, a comparison was made with the control series of 386 patients previously mentioned. Ideally, in a study of prognosis, the length of life should be determined from the date of onset of the disease. In the case of cirrhosis of the liver this is impossible because the early symptoms may be vague or entirely masked. Moreover, the patient, disabled by his disease, is unable to recall details of the beginning of his illness, which may have preceded the mature process by years. For these reasons it is more appropriate to select an objective sign, like ascites, which permits fairly easy and accurate recognition by the patient.

Since the patients at the Research Service were transferred from other hospitals, it is possible that the treated series fails to include certain patients

who would have died shortly after the onset of liver failure. In this way a bias would be introduced into the results. For example, in the control series 50 patients died and 16 patients were dropped from observation within 1 month of the onset of ascites. No such loss occurred in the treated series. In comparing the survival of the two series of patients, allowance was made for this degree of selectivity by excluding those patients who died or were "lost" within the first month.

We are indebted to Mr. Herbert H. Marks of the Metropolitan Life Insurance Company for the following analysis of the data:

A survivorship table was prepared, both for the treated cases and the controls, classified by individual months from the second through the eighth month, and by broader time groups beyond, namely for the ninth to twelfth months combined and for the second year. The experience past the second year is too small to be reliable. The experience for the first month was excluded from both series because the treated series consists very largely of cases referred from other hospitals and only a much smaller percentage were actually observed within a month of onset of ascites than in the control series. Elimina-

tion of this first month after onset of ascites largely reduces a bias in favor of the treated group.

The survivorship data are shown in detail in Table IV and the table has been so annotated that the method of computation can be followed. The last column of the table is presented graphically in Figure 1. Attention should be called to the fact that the method is approximate only, but the nature of the material and the small size of the samples do not warrant more refined analysis.

The greater longevity of the treated series is clear from the chart. Thus, the proportion surviving at the end of 6 months was 72 per cent of the treated series, compared with 57 per cent in the controls. At the end of the first year, 57 per cent of the treated group survived, but only 39 per cent of the controls. At the end of the second year, these ratios were 45 per cent and 21 per cent, respectively.

Mortality rates likewise were computed from Table IV. These are shown graphically in Figure 2. It is evident that during the first and second years after the onset of ascites the mortality rates were definitely lower in the treated series than in the controls.

TABLE IV

*Survival of patients with cirrhosis of the liver from onset of ascites*

(Comparison of cases treated at Research Service from August 1936 to November 1940, with a control group from several hospitals, from 1920 to 1940.)

TREATED SERIES						
Period*	Number observed at beginning of period	Died during the period	Dropped from observation during the period	Proportion dying during the period of those observed at beginning of period ( $q$ )	Proportion living at end of period of those observed at beginning of period ( $p = 1 - q$ )	Proportion surviving at end of period of original cohort ( $I$ )
	A	B	C	$D = B/A$	$E = 1 - D$	F
2nd month.....	47	1		0.021	0.979	0.979
3rd month.....	46	2		0.043	0.957	0.937
4th month.....	44	3		0.068	0.932	0.873
5th month.....	41	3		0.073	0.927	0.809
6th month.....	38	4	1	0.105	0.895	0.724
7th month.....	33	2		0.061	0.939	0.680
8th month.....	31	1	1	0.032	0.968	0.658
9th-12th months..	29	4	1	0.138	0.862	0.567
2nd year.....	24	5	6	0.208	0.792	0.449
CONTROL SERIES						
2nd month.....	230	41	15	0.178	0.822	0.822
3rd month.....	174	21	12	0.121	0.879	0.723
4th month.....	141	8	8	0.057	0.943	0.682
5th month.....	125	10	4	0.080	0.920	0.627
6th month.....	111	10	7	0.090	0.910	0.571
7th month.....	94	9	2	0.096	0.904	0.516
8th month.....	83	6	2	0.072	0.928	0.479
9th-12th months..	75	14	15	0.187	0.813	0.389
2nd year.....	46	21	4	0.457	0.543	0.211

\* First month after onset of ascites excluded.

It may be repeated that both the control and treated series are derived from the same social-economic levels; that the average ages of the two groups are the same; that the same criteria for diagnosis were applied to both series of patients; that the degree of liver failure as judged by symptoms and signs in Table I corresponds closely in the two series. However, in statistical comparisons of this kind there are uncontrolled factors which tend to favor one or the other group. For example, the control series of cases was taken from records over a 20-year period. It is possible

that the character of the disease has changed in recent years. The period of hospitalization is longer in the treated than in the control series. Despite these sources for error, the differences between the control and treated series appear to be significant.

*Prognosis after entry to the hospital.* There is one further serious source of error inherent in the statistical methods employed above. The accuracy of the data depends on the accuracy of the patient's definition as to when ascites formation began. Therefore, it seemed advisable to compare the two

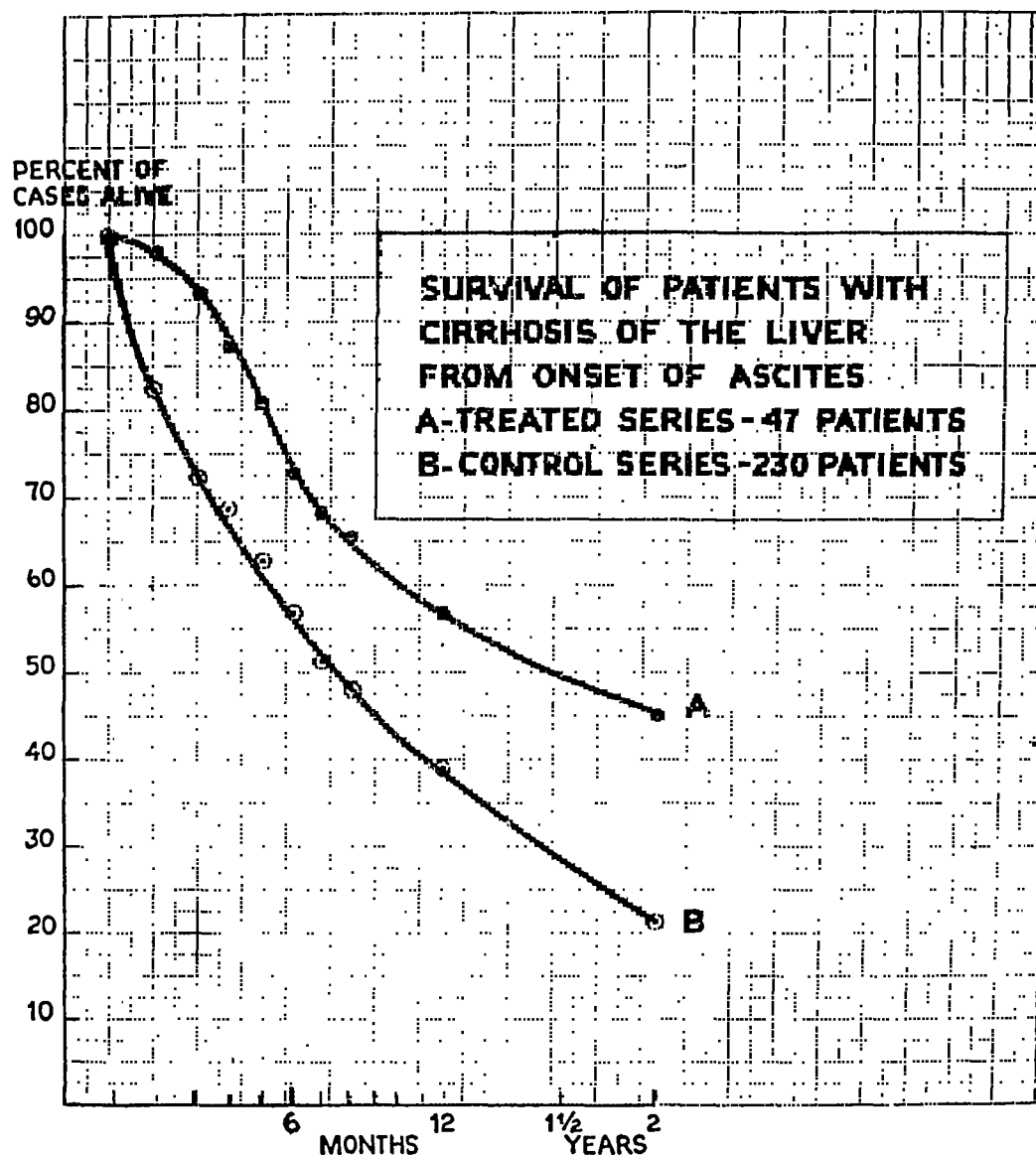


FIG. 1. SURVIVAL OF LIFE AFTER THE ONSET OF ASCITES IN PATIENTS WITH CIRRHOSIS OF THE LIVER

The proportion surviving at the end of each period (Table IV, column *F*) is multiplied by 100 to convert to the per cent of cases surviving. Computations beyond the second year are not used because the number of cases beyond that point in the treated series is too small.

## MORTALITY RATES IN CIRRHOSIS OF LIVER

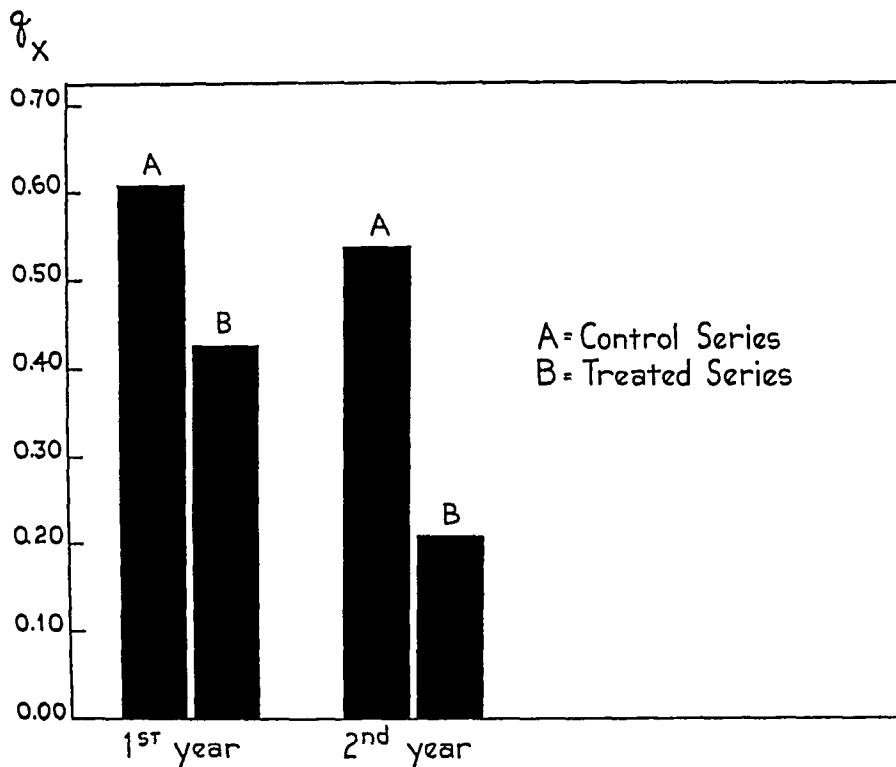


FIG. 2. DEATH RATES ( $q_x$ ) PER ANNUM IN CIRRHOSIS OF THE LIVER DURING THE FIRST AND SECOND YEARS AFTER THE ONSET OF ASCITES  
Comparison of control series and treated series.

series of patients from dates of first actual observation, when they were under medical supervision. Figure 3 illustrates the duration of ascites before and after entry to the hospital in the series of 47 treated patients. It is evident that about one-half of these patients lost ascites after treatment in the hospital. Several patients, whose ascites had disappeared for considerable periods of time and who had made clinical recovery from cirrhosis, died from intercurrent illness or accident.

Figure 4 illustrates the duration of ascites before and after entry to the New York Hospital<sup>4</sup> of 102 unselected patients with cirrhosis of the liver and ascites. (These form part of the control series of 386 cases.) In contrast to the treated series, there are less instances of remission from ascites in the control group. In 3 of the 7

instances\* in which ascites disappeared, the patients had received vitamin B concentrates. Likewise, the period of survival after entry to the hospital appears to be significantly less in the control group than in the treated series of 47 patients.

## DISCUSSION

*Comparison of mortality data in medical literature*

There are few data available in the medical literature which provide a norm for comparison with the present series of cases. In earlier reports cited by Patterson (33) and by Chapman, Snell, and Rowntree (34), the average life expectancy after the onset of ascites varied from 1 to 6 months. In a series of 38 cases Rolleston and McNee (35) reported an average life expectancy of 3 to 4 months after the onset of ascites; whereas Henrikson (36) reported an average duration of 12.8 months in a series of 42 patients

<sup>4</sup> We are indebted to Dr. Eugene Dubois for permission to study these case records.

\* Spontaneous loss of ascites occurred in 7 per cent of 386 cases.



# 47 PATIENTS WITH CIRRHOSIS OF THE LIVER AND ASCITES TREATED SERIES

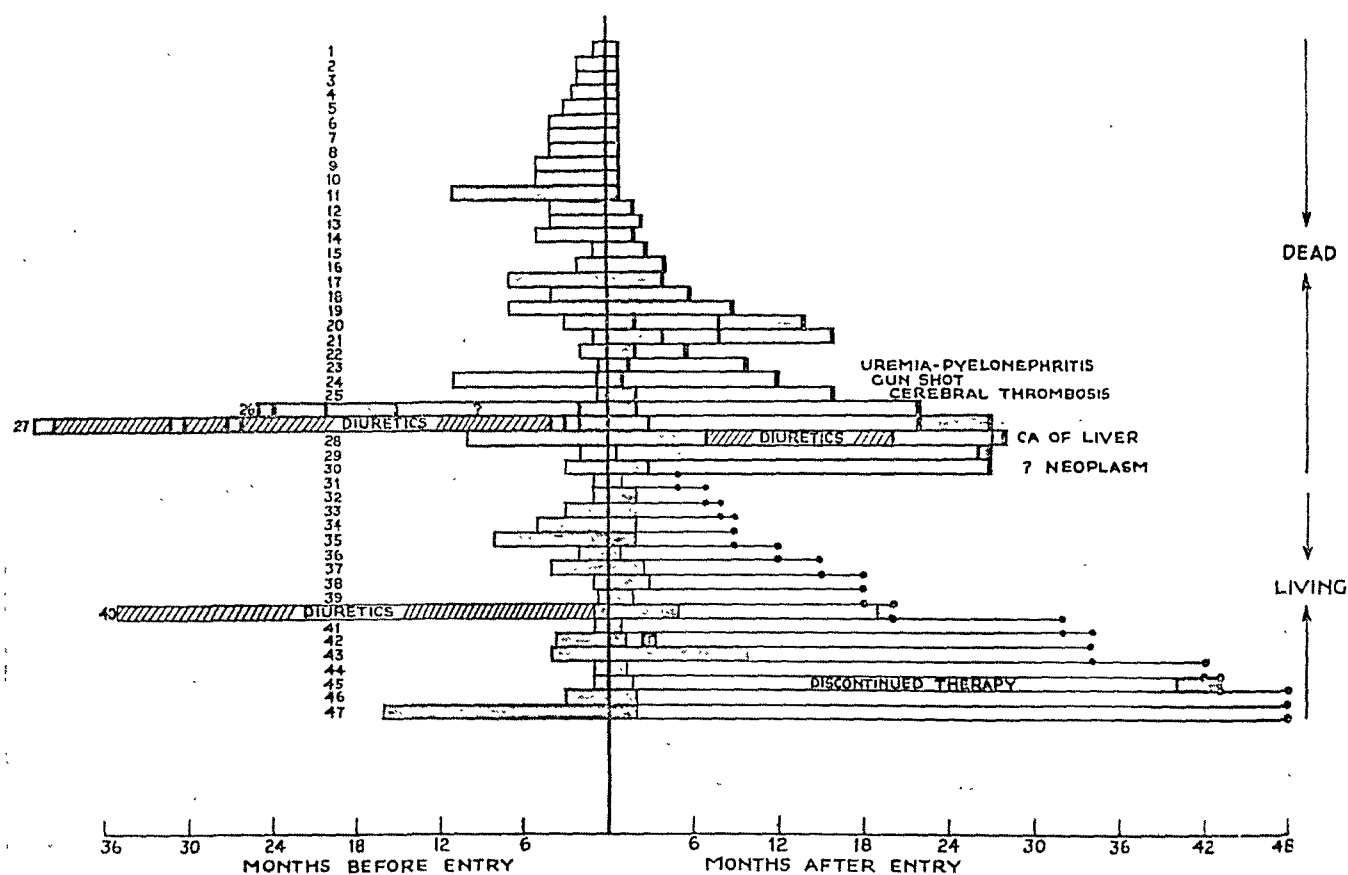


FIG. 3. CLINICAL COURSE OF 47 PATIENTS WITH CIRRHOSIS OF THE LIVER AND ASCITES (TREATED SERIES)

The central black line denotes the date of entry to the hospital. Time preceding entry is represented by columns to the left of this line, whereas time after entry is represented by columns to the right of the line. Shaded areas indicate the presence of ascites. The closed columns indicate a fatal issue. The open columns indicate that the patient was alive at the time of this analysis.

receiving no specific therapy. In his analysis of 372 cases Eppinger (37) gave no data on prognosis, but he stated that once abdominal paracentesis was performed, a fatal outcome was imminent. In contrast to this rather gloomy picture are numerous case reports (33, 34, 38, 39) of spontaneous recoveries, in which patients lived years after the disappearance of ascites. Although these are of interest, they are of limited value from a statistical point of view. Moreover, it is doubtful that recovery from a disease process occurs "spontaneously." Doubtless a cause exists for this change.

Because of the wide differences in the medical literature concerning the natural history of the disease, our treated series was compared only with our own control series.

## Other forms of treatment

The treatment of cirrhosis of the liver by other medical measures and by certain surgical procedures has been advocated. Chapman, Snell, and Rowntree (34) reported on the use of saline and mercurial diuretics in 84 of 112 carefully selected patients with decompensated portal cirrhosis, all of whom had ascites. The average duration of life after ascites appeared was 16 months. Twenty-five of these patients survived an average of 32 months after the onset of ascites. Jones and Eaton (40) have reported on the favorable effect of glucose infusions and a high carbohydrate diet in 50 patients with disease of the liver. The majority of their patients represented acute or subacute hepatitis, in which jaundice was the prominent feature. In 10 instances ascites was present.

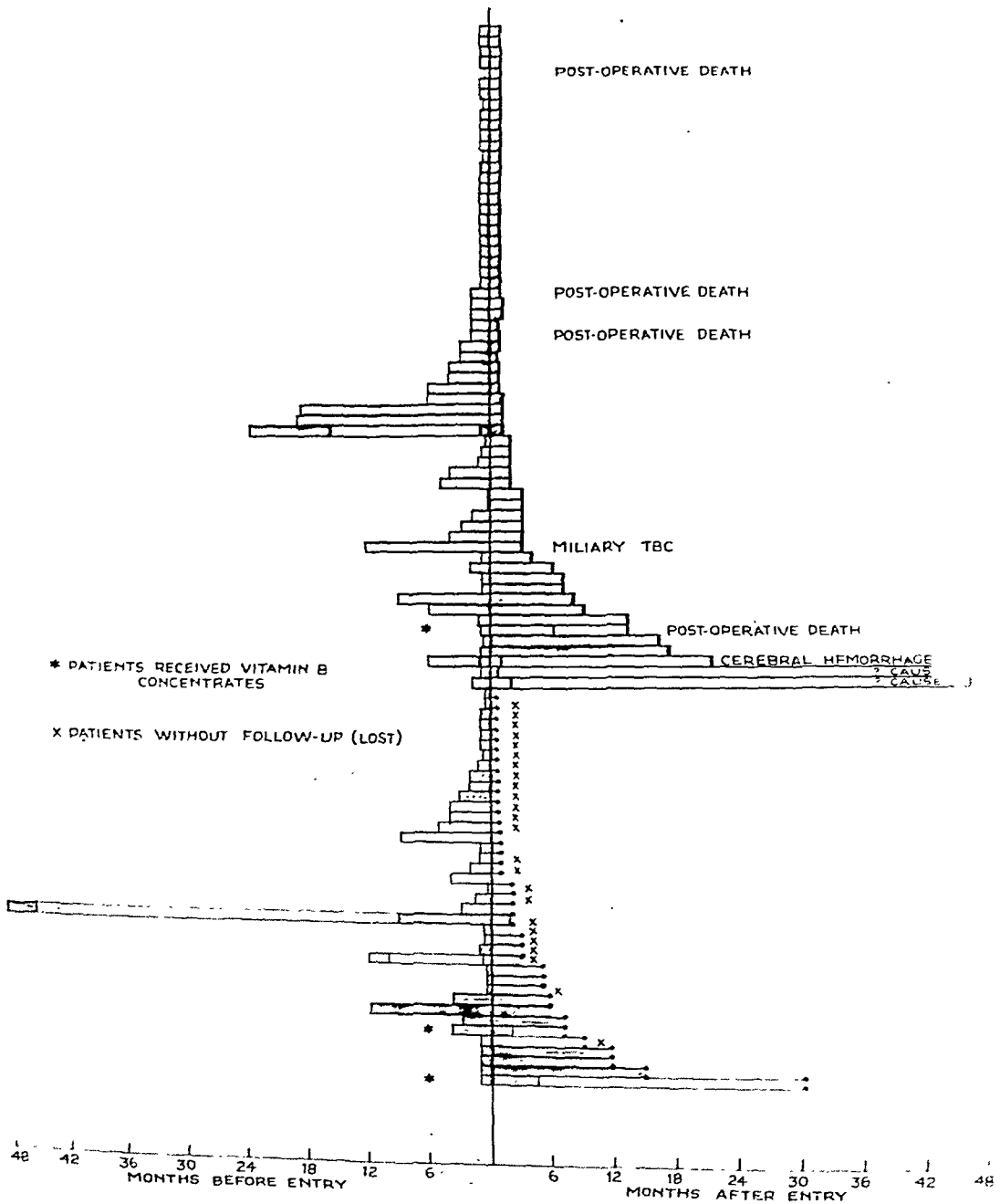
102 PATIENTS WITH CIRRHOSIS OF THE LIVER AND ASCITES  
CONTROL SERIES

FIG. 4. CLINICAL COURSE OF 102 PATIENTS WITH CIRRHOSIS OF THE LIVER AND ASCITES (NEW YORK HOSPITAL—CONTROLS)

The symbols correspond to those of Figure 3.

Because of differences in the selection of cases and in the types of cases studied, it is not possible to compare the relative merits of the various therapeutic measures with the present dietary regimen. Henrikson (36) has pointed out similar difficulties in evaluating surgical procedures that have been recommended for treatment of this disease. It is self-evident that the usefulness of one type of treatment does not exclude the merits of the others.

The use of liver preparations in the treatment of cirrhosis of the liver has been reported in the French medical literature (41, 42, 43) on the basis of substitutive organotherapy, "opothérapie." The data in these reports are incomplete and they are therefore difficult to evaluate. It is the impression of Rolleston and McNee (35) that a diet rich in milk and eggs is beneficial, although no data to substantiate this opinion are at hand.

In a report on alcoholic polyneuritis, Goodhart and Jolliffe (44) stated that 2 of 6 patients with cirrhosis of the liver lost ascites following the administration of vitamin B concentrates. Wayburn and Guerard (26) report a similar experience in 1 patient. A dietary program similar to that described in the present paper has been used at the Mayo Clinic during the past 2 years. Snell (45) reports that about one-third of a series of 50 patients with cirrhosis of the liver has shown considerable improvement.

#### *Interpretation of data*

The present treatment is based on the hypothesis that there is a significant relation between nutritional deficiency and the development of cirrhosis of the liver. However, there are two objections to this hypothesis. *First*, although signs of nutritional deficiency are often present in patients with cirrhosis of the liver, the incidence of cirrhosis in patients with deficiency diseases is not necessarily high. It is possible that, in fatal beriberi, pellagra, and sprue, patients die before cirrhosis of the liver can develop. Not infrequently the livers of such patients at autopsy show degenerative changes (48, 49). The observation that liver damage may occur in these diseases is supported by our experience with 24 patients admitted to the hospital primarily for nutritional deficiency. Of these, 22 were alcoholics with polyneuritis; 3 also had pellagra and 2 had sprue. Twenty of the 24

patients showed evidence of liver injury by physical signs or laboratory tests. In 10 of 15 patients treated with the dietary regimen previously described there followed partial or complete disappearance of these signs of liver dysfunction. *Second*, the signs of nutritional deficiency may be the result of interference with nutrition by the diseased liver. There is little doubt that this occurs. Studies on the metabolism of vitamin A (50) and vitamin K (51), for example, indicate that cirrhosis of the liver may interfere with the utilization of these vitamins. However, in many instances our patients gave histories of deficiency disease long antedating the symptoms or signs of cirrhosis. From these observations it seems likely that nutritional deficiency precedes the development of cirrhosis, and that the cirrhotic process, once established, tends to perpetuate or even aggravate the state of nutritional deficiency.

There are several further questions that present themselves in the interpretation of data submitted in this report: (1) Are the criteria for clinical improvement in cirrhosis of the liver adequate? (2) Granted that improvement takes place in certain patients, does this result from hospitalization with the removal of the patient from his former environment, or does it result from specific therapy? (3) If clinical improvement takes place, is there corresponding change in the liver structure? (4) What is the mechanism of "recovery"?

The evidence for clinical improvement in patients with cirrhosis of the liver is inadequate, since there are few signs or tests which may be used quantitatively for this purpose. It is difficult to separate signs of general bodily improvement from those that pertain to the function of the liver alone. Although certain tests are useful for the diagnosis of acute hepatitis, such as the galactose-tolerance and hippuric acid tests and the level of serum cholesterol, these have been of little help, in our experience, in gauging the presence or degree of liver cirrhosis. On the other hand, changes in the value for serum albumin correspond in most instances with changes in the clinical condition of the patient (31). The cephalin flocculation reaction likewise has reflected corresponding clinical changes. The bromsulfalein and Takata-Ara tests are useful as qualitative rather than quantitative measures.

In this report stress is laid on the disappearance

of ascites as a sign of improvement. It is realized that the disappearance of a sign of liver failure does not necessarily indicate a remission in the underlying disease process. However, the statistical evidence for decreased mortality rate, and the clinical evidence for bodily improvement in these patients, support the impression that the disease process has been modified. Admittedly, the criteria for improvement are inadequate, but to the clinician and to the patient the improvement is no less real.

There is little doubt that hospitalization and the removal of the patient from his former environment are beneficial. However, a control group of 386 patients who received hospital care does not show a comparable trend toward improvement. The two groups are not strictly comparable since the larger series had less prolonged hospital care. It is perhaps more significant that 4 patients with cirrhosis of the liver, who previously had an alcoholic background and who had shown signs of liver failure, were given 9 ounces of 40 per cent alcohol daily for from 6 to 18 months with no untoward effects. It may be argued justly that the amounts of alcohol administered were relatively small, according to the patients' habitual intake. Of necessity, this point could not be tested fully.

There is little, if any, evidence from the present data of correlation between clinical and histological changes in the liver. In the patients who died, presumably of liver failure, the histological picture of the liver showed wide differences. Within broad limits, it was impossible to tell the degree of liver competence by the histological picture. Similar difficulties with biopsy material are reported by Cates (46). In 2 patients who showed partial improvement for over 2 years, and who then died (Cases 28 and 29), there was marked necrosis of liver cells and periportal fibrosis similar in degree to the cirrhosis of patients who died after a rapid failure of several weeks. In 3 patients (Cases 23, 24, and 25) who had signs of liver failure with ascites on entry, and who made more or less complete clinical recovery but later died of non-hepatic lesions, the degree of cirrhosis at autopsy appeared to be minimal. Since biopsies were not done at entry, one can only speculate on anatomical changes that may have occurred. If anatomical changes were found, the evidence would be sug-

gestive. The absence of anatomical change would not imply necessarily a lack of functional change.

It is realized that cirrhosis of the liver often is discovered at autopsy in persons dying from other causes. In these cases cirrhosis may have been latent for many years. In a series of 245 autopsies that showed cirrhosis of the liver, McCartney (47) found that 35 per cent had no previous clinical manifestations of the disease. The present series obviously does not include latent cases, but treats only of those who showed evidence of failure of the liver during life.

The present data give no indication as to whether vitamin B concentrates, protein, or some unknown substances are specifically effective in the treatment of cirrhosis. It is possible that, by maintaining patients in optimal nutrition and rest, the liver recovers "spontaneously". In other words, the present regimen may allow the patient to survive long enough for natural reparative processes to take effect. It is also possible that the present regimen provides a specific agent for recovery of the diseased liver. The fact that only a limited number of patients survive despite therapy does not rule out the latter possibility. When the diagnosis of cirrhosis of the liver can be made in the incipient stages, the mortality rate should become much reduced for, unlike most other vital organs of the body, the liver has the capacity to regenerate. In this capacity lies the hope for an effective therapy.

#### SUMMARY

In summary, the present studies indicate that the treatment of cirrhosis of the liver by a highly nutritious diet supplemented with vitamin B concentrates is of therapeutic value. This is inferred from a comparison of 54 patients so treated with a control series of 386 patients with cirrhosis of the liver. Following the onset of ascites, 72 per cent of the treated series, as compared with 57 per cent of the controls, survived 6 months. At the end of the first year, 57 per cent of the treated series survived, but only 39 per cent of the controls. At the end of the second year, 45 per cent of the treated series, as compared to 21 per cent of the controls, were still alive.

In addition to an increased period of survival, there are signs of general bodily improvement and presumptive evidence of arrest of the disease

process. In a significant number of patients there has been disappearance of ascites, edema, jaundice, and of vascular "spiders". Certain laboratory data, such as the level of the serum albumin and of the serum globulin, the Takata-Ara test, cephalin flocculation test, and bromsulfalein dye test, have reflected corresponding improvement.

From the above evidence it seems clear that cirrhosis of the liver is not necessarily committed to a continuous, progressive course.

Since the treatment of the 54 patients involved a number of factors, the improvement may not be attributed to a specific agent.

The coexistence in these patients of nutritional deficiency and cirrhosis of the liver is striking. However, the relation of nutritional deficiency to cirrhosis is not clear. Certain experimental studies as well as the present clinical observations suggest that nutritional deficiency predisposes to the development of cirrhosis of the liver, and that cirrhosis of the liver, once established, tends to perpetuate the state of nutritional deficiency.

The etiologic relationship, if it exists, between nutritional deficiency and cirrhosis of the liver could be either direct or indirect. It is possible that the lack of certain food factors leads directly to the development of cirrhosis. It is also possible that the malnourished liver becomes vulnerable to toxins which ordinarily would be disposed of.

#### CONCLUSIONS

1. Evidence is presented which suggests that there is a significant relationship between the occurrence of nutritional deficiency and cirrhosis of the liver.

2. Fifty-four patients with cirrhosis of the liver were treated with a highly nutritious diet supplemented by vitamin B concentrates. The clinical course of the patients appeared to be more favorable than that of a control series of 386 hospitalized patients with cirrhosis of the liver.

#### CASE HISTORIES<sup>5</sup>

##### Group 1

*Case 31.* A 29-year-old Italian machinist entered the hospital on February 14, 1939, because of abdominal

swelling. Seven years previously pallor, weakness, and tarry stools had been observed. Three years previously jaundice had been present for several weeks. One month before admission there was sudden mid-abdominal pain, nausea, and vomiting for 1 day, followed abruptly by onset of ascites and jaundice. There was no response to diuretics. After one abdominal tap, ascites recurred. Exploratory operation was performed 2 weeks later. Liver and spleen were enlarged. Biopsy showed portal cirrhosis. One abdominal tap was performed after operation. Chief findings were: ascites, edema, palpable liver and spleen, vascular "spiders"; RBC 4.4, Hgb. 71%, WBC 3,500, serum albumin 3.3, serum globulin 3.2, bromsulfalein 25 to 30%, icteric index 6.

Dietary therapy was begun after operation. There was steady gain in weight and strength; ascites and edema disappeared in 1 month; serum albumin rose to 4.3; bromsulfalein remained 30%. The patient left against advice on June 29, 1939, 4½ months after entry. He moved from the city and there was no follow up.

*Case 24.* A 52-year-old colored waiter entered the hospital on April 28, 1937, because of abdominal swelling. He was a chronic alcoholic. A large liver had been noted 1½ years previously and 1 year before admission onset of ascites had occurred. He received eight abdominal taps at about monthly intervals preceding entry. Edema of legs was present for 2 weeks. There was no jaundice. His usual weight was 180. Chief findings were: ascites, edema, palpable liver and spleen; RBC 4.5, Hgb. 87%, WBC 9,400, serum albumin 3.2, serum globulin 3.8, Takata +, bromsulfalein 40%, icteric index 5. No neurological changes.

After entry, abdominal tap was done with removal of 11,000 cc. of fluid. Dietary therapy was then begun. Massive diuresis occurred during succeeding 2 weeks. Weight fell from 191 to 149 lbs. in 1 month and returned to 165 lbs. in the following 2 months without recurrence of ascites or edema. The patient left the hospital against advice on September 24, 1937, 5 months after entry. At this time serum albumin was 4.7, globulin 3.7, Takata negative, bromsulfalein 16%. He was seen at home on December 10, 1937, when he felt well. Liver was palpable but spleen was not felt. There was no ascites. He admitted drinking whiskey. Serum albumin at this time was 4.9, globulin 3.3; bromsulfalein 40%. He died from a gun shot wound on March 2, 1938. Gross changes at medico-legal autopsy showed thickened capsule of liver and spleen. No further description was available.

*Case 37.* A 36-year-old American-born stenographer entered the hospital on July 3, 1939, because of abdominal swelling. She had had typhoid at 10 years; was a chronic alcoholic. Her diet was poor in meat and dairy foods. She had had paresthesias in legs for 5 years, anorexia and severe pains in legs for 2 years. One and one-half years previously she had stopped work and had entered the hospital for 3 weeks. Diagnosis was "portal cirrhosis and alcoholic neuritis". Four months previously onset of ascites and edema had occurred. Jaundice, nausea and vomiting had been present for 3 months. She re-entered the hospital for 1 month before transfer

<sup>5</sup> When it is not specifically stated, the dietary regimen was begun after entry of the patient to the Research Service.

to Research Service. Two abdominal taps were performed. Her best weight was 137; her weight on admission was 119. Chief findings were: jaundice, ascites, edema, palpable liver and spleen, collateral vessels, vascular "spiders", dementia, polyneuritis, glossitis, scaly dry skin and hemorrhoids; RBC 2.7, Hgb. 51%, WBC 11,000, serum albumin 2.4, globulin 3.5, Takata +, icteric index 35, bromsulfalein 64%.

The patient received four abdominal taps in 2 months. Thereafter, she showed steady improvement, gained in strength and became mentally lucid. A mild fever subsided after 3 months. There was a gain of weight from 119 to 137 in 9 months without recurrence of ascites. Liver and spleen were smaller but palpable. The "spiders" disappeared. Icteric index was between 5 and 9. She was discharged from the hospital on April 2, 1940 and resumed an active, normal life. On October 23, 1940 (1½ years after entry) RBC 4.0, Hgb. 96%, WBC 7,500, serum albumin 4.7, globulin 2.9, Takata negative, bromsulfalein 32%, icteric index 5. She felt entirely well.

*Case 22.* A 36-year-old Irish American factory worker entered the hospital on August 7, 1937, because of vomiting blood. She had a history of alcoholism, "chronic indigestion", inadequate diet. Episodes of nausea and vomiting had occurred 2 to 3 times yearly during the previous 7 years. Blood-containing vomitus had been noted on several occasions. She had first entered the hospital June 1937 because of hematemesis and ascites. After two transfusions and one abdominal tap the patient left the hospital; 12 hours later, after another hematemesis, she entered the Research Service. Chief findings were: air hunger, pallor, jaundice, ascites, edema, vascular "spiders", glossitis, palpable liver and spleen, absent ankle jerks; temperature 102° F., pulse 130, blood pressure 90/50; RBC 1.78, Hgb. 31%, WBC 13,000, serum albumin 1.8, serum globulin 3.5, Takata +, icteric index 20, bromsulfalein 40%, cholesterol 265.

The patient was given a glucose infusion followed by transfusion immediately after entry. Temperature ranged from 102° to 104° F. for 3 days. For 6 weeks temperature was 100° F. and thereafter was normal. There was steady improvement for 5 months characterized by gain in strength, disappearance of jaundice, ascites, and edema. On December 3, 1937, laboratory data were: RBC 3.78, Hgb. 79%, WBC 7,700, albumin 4.0, globulin 3.7, icteric index 10, bromsulfalein 35%, Takata +. The patient felt well, left against advice, and resumed drinking habits. She returned 5 days later in an alcoholic stupor. Shortly after entry she had a massive hematemesis and died. Autopsy showed intestines full of blood. Liver weighed 2,000 grams and was finely nodular. Spleen weighed 340 grams. Histologic section showed portal cirrhosis.

*Case 38.* A 56-year-old Irish American housewife entered the hospital on March 8, 1939, because of vomiting blood. She had a history of alcoholism and poor diet; epilepsy for 35 years. Anorexia and periodic upper abdominal pain had been present for 1 year. Four months previously she had had hematemesis; 3 months

previously edema of ankles; 3 weeks previously onset of jaundice; 2 weeks previously hematemesis and onset of ascites. Chief findings were: semi-stupor, fever (laryngitis), jaundice, ascites, edema, palpable liver and spleen, vascular "spiders", collateral vessels, glossitis, polyneuritis, hemorrhoids, incontinence of urine and feces; RBC 2.3, Hgb. 45%, WBC 2,280, serum albumin 2.1, serum globulin 3.9, Takata +, icteric index 35, bromsulfalein 100%.

Abdominal taps were performed eight times in first 3 months. In the following 3 months diuresis was followed by disappearance of ascites and edema. Her weight fell from 163 to 140 lbs. with diuresis. She became mentally clear and gained in strength. Jaundice cleared and vascular "spiders" disappeared. Six months after entry she was ambulatory and strong. She left hospital against advice on September 11, 1939, and was seen in O.P.D. 2 months later. Liver and spleen were palpable. No ascites, edema, or jaundice was present. Laboratory data were: RBC 4.0, Hgb. 75%, WBC 9,400, serum albumin 4.0, globulin 3.5, Takata +, bromsulfalein 53%, icteric index 7. On September 17, 1940, she returned to the clinic (1½ years after entry to hospital). Except for anemia (hemorrhoidal bleeding), there was no clinical change. Laboratory data were as follows: RBC 2.5, Hgb. 52%, serum albumin 3.7, globulin 3.4, bromsulfalein 26%, icteric index 5. She felt well.

*Case 23.* A 48-year-old Irish housewife entered the hospital April 2, 1937, because of vomiting and abdominal fullness. There was a past history of alcoholism, of 15 pregnancies (9 miscarriages) and of a diet very poor in meat and dairy products. Vomiting attacks had been occurring 1 to 2 hours after meals for 1 year. Her weight loss had been 40 lbs. in 1 year. She had had abdominal distention for 4 months; ascites for 2 weeks; increasing weakness, dyspnea with exertion. Her best weight had been 150; her recent weight was 92. Chief findings were: pallor, emaciation, mental torpor, nystagmus, glossitis, dry scaly skin, palpable liver and spleen, edema, absent knee and ankle jerks; RBC 1.2, Hgb. 34%, M.C.V. 154, WBC 3,000, albumin 2.2, globulin 3.5, Takata +, bromsulfalein 40%, gastric analysis 0 free HCl, Wassermann +, urine albumin trace, many WBC, NPN 55.

The anemia did not respond to liver therapy. During the first month she had daily vomiting and one hematemesis; the ascites increased. On May 10, 1937, she had transfusion with 500 cc. blood. Improvement followed with a gain in weight and strength. Vomiting ceased; diuresis with loss of ascites occurred after 6 weeks; the liver edge receded. She became mentally clear and felt well. She was discharged against advice on October 6, 1937, 6 months after entry. At this time laboratory data were: RBC 2.86, Hgb. 58%, WBC 5,000, serum albumin 3.7, globulin 3.0, Takata negative, icteric index 4, bromsulfalein 10%. Urine showed albumin and many WBC, NPN 74 (at entry 55). She was readmitted 4 months later (February 5, 1938) because of vomiting and nose bleeds. Chief findings were: pallor, stupor, palpable liver and spleen, blood pressure 170/120, pericardial rub;

P.S.P. test 1% in 2 hours, NPN 400, urine as before, no ascites, no jaundice. The patient died in uremia after 1 week. Autopsy showed pyelonephritis; cirrhosis of the liver (slight fibrosis).

*Case 41.* A 55-year-old American salesman entered the hospital on December 28, 1937, because of abdominal swelling. He had a history of alcoholism. His diet had been poor in meat and dairy products. He had periodic nausea and diarrhea. Twelve years previously he had been hospitalized for 3 months because of hypertensive heart disease. Two years before admission he had been hospitalized for 2 months because of jaundice and edema; there had been a question of ascites. At that time the blood pressure was 180/100; liver was palpable; urine showed RBC, WBC, albumin, granular casts. On admission chief findings were: palpable liver and spleen, ascites, edema, vascular "spiders", glossitis, neuritis; RBC 4.4, Hgb. 98%, WBC 7,000, Takata  $\pm$ , serum albumin 2.7, serum globulin 4.7, icteric index 5, bromsulfalein 40%, venous pressure 90, circulation time 12 seconds (decholin). Urine showed RBC, WBC, albumin and occasional casts; PSP test 80%, concentration up to 1020.

One abdominal tap was performed. Diuresis with loss of ascites occurred in 1 month. His weight fell from 268 to 212 lbs. There was no recurrence of ascites, edema, or jaundice. Liver remained palpable. There was steady gain in strength. Blood pressure was variable between 140/90 and 190/100. During the last 6 months of his hospital stay he received 9 ounces of 40% alcohol daily with no untoward effects. He was discharged on June 5, 1939, 1½ years after entry. At this time laboratory data were: RBC 5.3, Hgb. 105%, WBC 6,500, albumin 3.7, globulin 3.3, bromsulfalein 28%, icteric index 2, Takata negative. In O.P.D. on March 5, 1940, blood counts and urinary sediment were the same as before; serum albumin 4.4, globulin 2.8. The patient was active as salesman. On September 7, 1940 (3½ years after entry), findings were essentially unchanged; he felt well.

*Case 25.* A 46-year-old American seaman entered the hospital on November 16, 1937, because of vomiting. There was a history of typhoid 40 years before, of alcoholism for 25 years, and of a diet poor in meat and vegetables. Seventeen years previously a chancre had been treated with one course each of arsphenamine and tryparsamide. Nausea, vomiting, and abdominal distress had been present 10 years. Signs of tabes dorsalis were recognized for 7 years. Periodic edema of ankles had occurred for 4 years; severe nausea and vomiting for 2 weeks, followed by onset of jaundice, ascites, and edema. His best weight had been 149; his weight on admission was 117. Chief findings were: emaciation, jaundice, irregular fixed pupils, palpable liver and spleen, ascites, edema, tremor, absent knee jerks and ankle jerks, Romberg +, hemorrhoids, blood pressure 120/78; RBC 4.4, Hgb. 95%, WBC 5,800, Takata +, serum albumin 3.0, globulin 3.6, icteric index 15, bromsulfalein 45%, blood Wassermann +, spinal fluid negative.

Ascites formation increased 3 weeks after entry with

a gain in weight from 124 to 156 lbs. Dietary therapy and four injections of mercupurin were given. After 3 weeks diuresis with loss of ascites and edema occurred. There was no recurrence during next 1½ years. He gained in weight and strength. He had occasional "gastric crises" and "shooting pains" and a sudden hemiplegia on April 12, 1939. Blood pressure was 160/110; spinal fluid was bloody. He had a terminal bronchopneumonia and died May 4, 1939, 16 months after entry. Laboratory data on January 15, 1939 (14 months after entry) were: RBC 4.4, Hgb. 85%, WBC 9,400, serum albumin 4.8, globulin 2.1, Takata negative, bromsulfalein 8%, icteric index 4. With terminal illness, 3 months later, there was a rise in icteric index to 14 and in bromsulfalein to 44%. Autopsy showed tabes dorsalis, bronchopneumonia, cirrhosis of liver (minimal fibrosis). Autopsy on head was not granted.

*Case 30.* A 63-year-old French chef entered the hospital May 9, 1938, because of dyspnea and recurrent ascites. Past history was irrelevant. Right upper quadrant pain, headaches and dyspnea had been present for 4 years. Increasing dyspnea, palpitation and weakness had been present for 3 years. One year previously he had entered the hospital for 1 month. The diagnosis was hypertensive heart disease and enlarged liver. Blood pressure was 200/100. He had slight ankle edema. Onset of ascites had occurred 3 months before present admission. Two months previously he had entered another hospital. He vomited blood for a day after entry. X-ray showed esophageal varices. Digitalis and mercupurin were administered. Abdominal taps were performed weekly for 7 weeks. His weight loss had been 40 lbs. in 2 years. On transfer to Research Service, chief findings were: emaciation, dyspnea, cardiac enlargement, blood pressure 200/100, ascites, slight peripheral edema, palpable, non-tender liver and spleen, pigmentation, diminished vibratory sensation in legs; RBC 3.6, Hgb. 68%, WBC 8,800, Takata +, icteric index 3, bromsulfalein 18%, albumin 3.2, globulin 3.2; urine showed albumin + and many WBC. Right pyelogram showed hydronephrosis; venous pressure was 70, circulation time was 16 seconds (decholin).

He was maintained on digitalis, grains 1½ daily. He had six abdominal taps in the first 3 months and none thereafter for 2 years. Digitalis was withheld for 1 month on 3 occasions. Venous pressure rose to 150 mm., H<sub>2</sub>O and circulation time to 20 seconds without return of ascites or edema. It was therefore believed that this was not a case of cardiac cirrhosis. Pyuria cleared with mandalate therapy. The patient became ambulatory and well. On May 2, 1940, laboratory data were: RBC 4.2, Hgb. 82%, WBC 6,000, albumin 4.3, globulin 3.5, Takata negative, bromsulfalein 20%. He was readmitted on July 13, 1940, with signs attributable to abdominal malignancy. There was no sign of heart failure and no return of ascites. He died on August 19, 1940. There was no autopsy.

*Case 43.* A 61-year-old Irish bartender entered the hospital January 7, 1938, because of abdominal swelling. There was a history of typhoid fever and malaria in

childhood, of gonorrhea 40 years before hospital entry, and of alcoholism for 25 years. Hemorrhoids were present for 6 years. Massive hematemesis, followed by intermittent abdominal distention, occurred 4 years before admission. A massive hematemesis occurred 3 years before, which necessitated a blood transfusion. Icterus was noted. He had cramps and paresthesias in legs for 1 year. For 4 months there had been anorexia and increasing abdominal fullness. For 2 months he had noticed swelling of ankles and feet and had felt weak. His average weight was 180. Chief findings were: ascites, edema, palpable liver and spleen, vascular "spiders", collateral veins, pigmentation, umbilical hernia, polyneuritis, mental confusion, glossitis, hemorrhoids, urethral stricture; RBC 4.3, Hgb. 92%, WBC 2,800, Takata +, icteric index 5, bromsulfalein 40%, serum albumin 3.2, serum globulin 3.5.

Fifteen abdominal taps were performed in 10 months with time intervals between taps gradually lengthening. During the next 4 months there was no recurrence of ascites or edema. Liver and spleen were barely palpable. He had surgical repair of strangulated umbilical hernia on January 12, 1939, without ill effect. He was discharged April 15, 1939 and resumed work. He had a repair of the urethral stricture on May 20, 1939. On follow-up August 21, 1940 (2½ years after entry), there was no clinical change. Laboratory data at this time were: RBC 4.6, Hgb. 90%, WBC 3,500, albumin 4.7, globulin 2.7, Takata negative, bromsulfalein 18%, icteric index 5. He felt well and was active as bartender.

*Case 47.* A 45-year-old Irish bartender's wife entered the hospital October 5, 1936, because of swollen abdomen. She had a history of "moderate" alcohol intake. Anorexia, nausea and vomiting, followed by onset of ascites and hematemesis, had occurred 1½ years before admission. She was hospitalized for 6 months and received mercurial diuretics and four abdominal taps. Three days after discharge she entered another hospital because of hematemesis. She had nine abdominal taps in the next 4 months and was transferred to Research Service on October 5, 1936. Chief findings were: emaciation, dry scaly skin, glossitis, vascular "spiders", collateral circulation, ascites, edema, palpable liver and spleen, clubbed fingers, right hydrothorax, net weight 93 lbs.; RBC 4.0, Hgb. 81%, WBC 7,500, serum albumin 2.2, globulin 2.8, bromsulfalein 24%, Takata +, icteric index 8.

The patient's condition was critical during the first 6 weeks. Two abdominal taps were performed. Thereafter, gradual diuresis with loss of ascites and edema occurred, followed by disappearance of vascular "spiders" and clubbing. Liver and spleen were smaller but still palpable. She showed striking gain in weight and strength and was ambulatory after 11 months. Her weight was 145 lbs. She was discharged January 18, 1938, 15 months after entry, and resumed housework. On August 23, 1940 (4 years after entry) the liver and spleen were still palpable, weight was 145 lbs.; RBC 4.5, Hgb. 88%, WBC 5,500, albumin 4.9, globulin 3.1, icteric

index 5, bromsulfalein 20%, Takata negative. She felt entirely well.

*Case 46.* A 63-year-old Irish laborer entered the hospital July 4, 1936, on account of abdominal swelling. He was an alcoholic and his diet had been poor in meat and dairy products. For 5 years nausea and vomiting had occurred after drinking liquor. For 1 year he had had intermittent edema of ankles. For 3 months he had had progressive swelling of the legs, scrotum, abdomen, and transient jaundice. Chief findings were: undernutrition, ascites, edema, palpable liver, collateral veins, glossitis, polyneuritis, hemorrhoids; RBC 4.6, Hgb. 99%, WBC 12,000, albumin 2.2, globulin 3.6, icteric index 4, bromsulfalein 25%, Takata +.

Four abdominal taps were performed in 2 months. He had a sudden diuresis followed by loss of ascites and edema. His weight fell from 160 to 120 lbs. in 3 weeks. Thereafter he showed a steady gain in weight and strength. The liver edge receded. The patient felt well and resumed former work. At the time of discharge on February 15, 1938, his weight was 145 lbs., RBC 4.8, Hgb. 95%, WBC 7,000, serum albumin 5.0, globulin 3.8, bromsulfalein 20%, Takata ±, icteric index 5. He returned to follow-up 10 months later (December 2, 1938) because of paresthesias in legs. There was no return of ascites or edema; liver was barely palpable; polyneuritis was marked. Vitamin therapy was resumed, together with 9 ounces of 40% alcohol daily for the ensuing 14 months. There was no essential change. The patient was ambulatory and well. On October 1, 1940 (4 years after first entry) laboratory data were as follows: RBC 4.3, Hgb. 84%, WBC 9,000, albumin 4.5, globulin 2.7, icteric index 7, bromsulfalein 25%, Takata negative.

*Case 42.* A 57-year-old Russian sailor entered the hospital January 14, 1938, because of abdominal swelling. He was an alcoholic and his diet had been poor in meat and vegetables. He had had hemorrhoids 2 years before admission. Onset of jaundice, ascites and edema had occurred 3½ months before admission. He had been hospitalized October 18, 1937, for 3 weeks. Examination showed above signs and an icteric index of 45. He had diuresis with mercurial drug. His weight fell from 205 to 173 lbs. He was readmitted on December 13, 1937, with identical signs. His icteric index was 18. He received an abdominal tap and mercurial diuretics and was transferred to Research Service. Chief findings were: ascites, edema, palpable liver and spleen, collateral veins, hemorrhoids, glossitis, polyneuritis; RBC 3.1, Hgb. 76%, WBC 4,500, serum albumin 2.8, globulin 4.5, icteric index 7, Takata +, bromsulfalein 40%, urine negative.

There was loss of ascites and edema during the first month and gain in strength. On February 15, 1938, he had an upper respiratory infection followed in 3 weeks by signs of acute nephritis with anasarca. Urine showed albumin, RBC's and casts. Blood pressure rose from 130/75 to 160/100; N.P.N. from 30 to 65; weight increased from 157 to 187 lbs. (anasarca); serum albumin fell from 3.1 to 2.0. Steady clinical improvement took place in 6 weeks with loss of ascites and edema. At dis-



charge, January 19, 1939 (1 year after entry), the laboratory data were: RBC 4.0, Hgb. 90%, WBC 4,100, albumin 3.8, globulin 3.0, bromsulfalein 30%, Takata negative, icteric index 7. He resumed work. He was readmitted April 14, 1939, with a transient jaundice after an upper respiratory infection. It subsided in 1 week and he resumed work. He was readmitted May 28, 1940, for study. He felt entirely well. Liver was not palpable; spleen was palpable; weight was 178. There was no ascites, edema, or jaundice and no change in laboratory data. On September 7, 1940 (2 years and 9 months after entry) there was no essential change.

*Case 45.* A 40-year-old negro painter entered the hospital January 26, 1937, because of jaundice and swollen abdomen. He had a history of alcoholism, chancre 15 years before, poor diet. One year previously he had entered the hospital for 6 months because of jaundice, edema and dyspnea. After discharge he resumed old habits. He had had increasing ascites, jaundice, abdominal pain, nausea and vomiting for 1 month before admission. He had had epistaxis 1 day. He entered the hospital December 16, 1936 and had an abdominal tap on the day of entry. Ascites returned. He had diuresis after 1 month with KI, saline and mercurial diuretics. On transfer to Research Service, the chief findings were: jaundice, vascular "spiders", palpable liver and spleen; RBC 3.9, Hgb. 76%, WBC 6,200, Wassermann 2+, serum albumin 3.6, globulin 3.8, icteric index 33, Takata +. No ascites or edema was present.

The patient had a chill and fever for 2 days. Temperature was 100 to 101 for 1 week; thereafter it ran a benign course. The liver edge receded. There was no return of ascites or edema. Jaundice disappeared. The patient was discharged after 8 months (October 9, 1937) with RBC 4.6, Hgb. 99%, WBC 6,200, albumin 3.9, globulin 2.7, icteric index 8, bromsulfalein 36%, Takata negative. He felt entirely well. He was symptom-free for 3½ years, then resumed previous drinking habits and was readmitted to the hospital in May 1940 with ascites and jaundice.

*Case 48.* A 49-year-old Irish American clerk entered the hospital November 27, 1936, because of swollen legs. She gave a history of chronic alcoholism and poor diet. Five years previously she had been hospitalized for syphilis, pulmonary tuberculosis and multiple neuritis. She received 15 injections of neoarsphenamine; her Wassermann became negative. The infiltration at the right lung apex healed. Biceps, triceps, Achilles reflexes remained absent. Paresthesias disappeared. Liver and spleen were not palpable. She was discharged July 28, 1932, 10 months after entry. In the interval before she re-entered the hospital alcoholism was severe and neuritis recurred. One month before re-entry she had transient jaundice and edema of legs. Chief findings were: obese woman, mentally dull and confused; tremor of hands, pellagrous dermatitis, glossitis, umbilical hernia, palpable spleen and liver, vascular "spiders", polyneuritis, phlebitis; RBC 3.5, Hgb. 88%, WBC 6,500, serum albumin 4.2, globulin 4.5, Takata +, bromsulfalein 10%, icteric index 3. No ascites or jaundice.

Jaundice cleared before entry. With therapy, there was marked improvement in psyche and sensory components of neuritis. The liver receded in size; the spleen was still palpable. Laboratory data on discharge were: bromsulfalein 0, albumin 4.5, globulin 3.1, Takata negative. Other laboratory data were unchanged. She was discharged April 1, 1937, 4 months after entry, and resumed work. There was no follow up.

*Case 49.* A 48-year-old American housewife entered the hospital March 13, 1937, because of jaundice. She gave a history of alcoholism and poor diet. Four years previously she had been jaundiced for several months. For 2 years she had been on a liquid diet and for 6 months she had had dysphagia and regurgitation of foods. She had been bedridden for 4 months because of weakness and paralysis. Jaundice recurred 1 month before admission. Chief findings were: Obese, jaundiced woman, dull, confused, disoriented; nystagmus, pellagrous dermatitis, glossitis, severe polyneuritis, vascular "spiders", palpable liver; RBC 3.46, Hgb. 77%, WBC 7,000, serum albumin 3.7, globulin 3.6, Takata negative, bromsulfalein > 40, icteric index 30. There was questionable ascites and splenomegaly.

The patient was fed by nasal catheter for 1 month and thereafter ate solid foods. She showed marked improvement in strength and psyche. There was a subsidence of neuritis, dermatitis and glossitis; the liver edge receded, the jaundice disappeared after 1 month. The patient became ambulatory but had a residual foot drop. She was discharged December 23, 1937, 7 months after entry. Laboratory data were: RBC 4.2, Hgb. 91%, WBC 6,000, icteric index 3, bromsulfalein 20%, albumin 4.9, globulin 2.8, Takata negative. On June 25, 1940, the patient was ambulatory and well. Laboratory data were essentially unchanged 3½ years after entry.

*Case 50.* A 49-year-old Irish American housewife entered the hospital November 1, 1938, because of weakness. She was a chronic alcoholic whose diet had been poor in meat and dairy foods. Nausea, vomiting, abdominal cramps had occurred for several years. One year previously she received 24 injections of neoarsphenamine. For 10 months there had been six loose stools daily, and for 1 month vomiting spells, inability to walk, and paresthesias of the legs were noted. Her best weight was 145; her weight on admission was 128. Chief findings were: mental torpor and confusion, nystagmus, polyneuritis, rough dry skin, vascular "spiders", palpable liver, collateral veins, glossitis, hemorrhoids; RBC 4.8, Hgb. 85%, WBC 5,000, serum albumin 3.6, globulin 3.6, Takata +, bromsulfalein 20%, icteric index 5. No jaundice or ascites was present.

The patient gained in weight and strength; the liver edge receded; the "spiders" disappeared. She had no G.I. disturbances. The neuritis was only partially improved. She was discharged on March 15, 1940 and resumed work. On discharge laboratory data were: RBC 4.5, Hgb. 96%, WBC 11,000, serum albumin 5.1, globulin 2.9, Takata negative, icteric index 5, bromsulfalein 0. On October 1, 1940, 2 years after entry, there was no clinical change. She felt well.

*Case 51.* A 53-year-old Irish American clerk entered the hospital October 19, 1939, because of numbness of legs. He gave a history of alcoholism and poor diet. He had been hospitalized February 1, 1939, because of optic neuritis. Chief findings were: optic atrophy, polyneuritis, palpable liver and spleen, collateral veins, glossitis; bromsulfalein 30%, albumin 4.3, globulin 3.3, Takata negative. There was no ascites. The patient was seen periodically in O.P.D. and was admitted to Research Service because of paresthesias in legs. Added to the above findings were icterus and vascular "spiders", RBC 4.6, Hgb. 86%, WBC 6,700, albumin 4.5, globulin 3.3, bromsulfalein 40%, icteric index 15, Takata negative.

The patient was placed on dietary therapy and was given 9 ounces of 40% alcohol daily during 6 months' stay. He showed steady gain in weight and strength. Liver and spleen remained palpable. Neuritis subsided. Vascular "spiders" disappeared. On discharge laboratory data were: RBC 4.8, Hgb. 90%, WBC 3,500, albumin 5.1, globulin 2.7, icteric index 7, bromsulfalein 16%, Takata negative. On October 1, 1940, 1 year after entry, there was no clinical change.

*Case 52.* A 26-year-old Irish American typist entered the hospital December 11, 1937, because of a mass in the abdomen. She gave a history of alcoholism and poor diet. She had had anorexia for 3 years, nausea and vomiting for 2 years, and a mass in the abdomen and edema of ankles, followed by severe epigastric pain and jaundice, 2 months before admission to the hospital. She was hospitalized November 4, 1937. In the hospital she had a hematemesis and epistaxes. Her icteric index was 16. Exploratory laparotomy revealed large, granular liver. Biopsy showed fatty degeneration and periportal fibrosis. Severe paresthesias of legs developed post-operatively. After 5 weeks patient signed release; she entered Research Service 2 weeks later. Her weight 8 years previously had been 127; 3 years previously 110; 1 month previously 80 lbs. Chief findings were: Emaciated, pale, hysterical young woman; dry, parchment-like skin, vascular "spiders", hard, tender liver filling right half of abdomen, palpable spleen, collateral veins, glossitis, polyneuritis, hemorrhoids; RBC 3.3, Hgb. 71%, WBC 7,250, Takata +, icteric index 2, bromsulfalein 5%, serum albumin 3.9, globulin 4.7. No ascites.

In July 1938, the patient had an episode of fever, chills, colon bacilluria due to left ureteral stone; symptoms subsided with passage of stone. In September 1938, she had transient jaundice for 2 weeks; no apparent cause. (Icteric index rose to 25.) Except for these 2 complications, she showed steady improvement. There were no G.I. disturbances; the liver edge receded; vascular "spiders" disappeared; neuritis subsided. The patient resumed work on December 5, 1938, 1 year after entry. She was seen periodically in O.P.D. and felt entirely well. Her weight was 123 lbs., the liver edge was at the costal border. On June 4, 1940, laboratory data were: RBC 4.5, Hgb. 85%, WBC 7,000, icteric index 5, bromsulfalein 10%, albumin 5.4, globulin 3.1, Takata negative. On September 7, 1940 (2 years, 9 months after entry), there was no clinical change.

*Case 44.* A 45-year-old Irish cook entered the hospital April 28, 1937, because of painful, weak legs. She gave a history of alcoholism and a diet poor in meat and vegetables. She had had a daily purgation with epsom salts for the previous 4 years. Her weight had fallen from 150 to 94 lbs. She had been hospitalized 5 years previously for alcoholism. She had had progressive weakness and paresthesias of legs for 5 months; dysphagia, incontinence of urine and feces for 3 months; persistent vomiting for 2 months. She was hospitalized March 31, 1937, and transferred to Research Service 1 month later. Chief findings were: pale emaciated, disoriented; skin rough, dry, and pigmented; vascular "spiders", palpable liver, ascites, glossitis, collateral veins, severe polyneuritis; RBC 3.7, Hgb. 81%, WBC 10,000, serum albumin 3.8, globulin 2.7, bromsulfalein 20%, Takata negative, icteric index 3. She had no jaundice.

Except for transient phlebitis of leg, improvement was steady. There was no recurrence of ascites; the liver edge receded; glossitis and polyneuritis subsided. Her weight rose to 140 lbs. In addition to dietary therapy, the patient received 9 ounces 40% alcohol daily for 1½ years without causing apparent change. She was discharged September 1, 1939, to resume work and was seen periodically in O.P.D. Laboratory data on May 18, 1940 were: RBC 5.0, Hgb. 95%, WBC 7,450, serum albumin 5.0, globulin 2.5, Takata negative, bromsulfalein 30%, icteric index 7. She felt entirely well 3 years after entry.

#### Group 2

*Case 26.* A 40-year-old Italian barber entered the hospital January 7, 1938, because of swelling of legs and abdomen. There was a past history of chronic alcoholism. He had had varicose veins 15 years previously and intermittent swelling of legs for 10 years. He had had phlebitis 3 years previously. Previous hospital admissions: (1) March, 1934: Edema, hematuria, albuminuria, palpable liver, B.P. 165/100. Diagnosis: nephritis; cirrhosis of liver. (2) July, 1935: Palpable liver and spleen, jaundice, clubbed fingers. (3) November, 1935: Palpable liver and spleen, jaundice, ascites. Urine negative. Loss of ascites with mercurial diuretics. (4) April, 1936: Ascites, edema, palpable liver and spleen, jaundice (icteric index 40), phlebitis. (5) November, 1938: Above findings. Diuresis with mercurial diuretics. Weight fell from 280 to 230 lbs. Transferred to Research Service. Chief findings were: Obese Italian; jaundice, vascular "spiders", palpable liver and spleen, ascites, umbilical hernia, thrombophlebitis of legs, clubbed fingers; RBC 4.8, Hgb. 84%, WBC 3,400, bromsulfalein >40%, icteric index 20, Takata +, serum albumin 2.4, globulin 4.8.

His chief disability was recurrent phlebitis; he had nine episodes with chill, fever, and painful swelling of legs; he had frequent hemoptyses. On at least two occasions he had a pulmonary infarction. Otherwise the patient felt well during his 18 months' stay. Ascites disappeared without recurrence. His weight fell from 230 to 210; albumin rose to 3.0, globulin fell to 3.8;

icteric index 10 to 15. There were no other changes and his course was considered stationary. He was discharged July 19, 1939, and died suddenly at another hospital 4 months later. There was question of a pulmonary embolus. There was no autopsy.

*Case 27.* A 49-year-old Italian laborer entered the hospital January 1, 1938, for abdominal swelling. He gave a history of alcoholism and poor diet; he had had syphilis and arsphenamine therapy 25 years previously. Previous hospital admissions: June, 1934: Jaundice, ascites; abdominal tap. April, 1935: Ascites, edema, palpable liver, hematemesis; abdominal tap. June, 1935: Ascites, edema, palpable liver and spleen, jaundice, glossitis, vascular "spiders"; abdominal tap. September, 1937: Signs and therapy as above. November, 1937: Above signs, vomiting, melena; icteric index 25. Between hospital entries, the patient received injections of mercurial diuretics 2 to 3 times weekly to control ascites. He entered Research Service on January 1, 1938. Chief findings were: Icterus, nystagmus, ascites, edema, vascular "spiders", pigmentation, glossitis, palpable liver and spleen; RBC 4.2, Hgb. 88%, WBC 7,200, albumin 3.2, globulin 4.0, icteric index 10, bromsulfalein > 40, Takata +; occasional RBC, WBC, casts and + albumin in urine. There was a question of polyneuritis.

The patient had four abdominal taps in 3 months; thereafter he was ambulatory and ascites-free for 1½ years without taps or diuretics. Takata was negative. In September, 1939 he had a sudden chill, fever and signs of pneumonia, with rapid onset of ascites. He had weekly abdominal taps until death, 7 months later. Albumin declined 3.6 to 2.8; icteric index rose from 6 to 15; bromsulfalein rose from 40 to 80%; spleen was enormous, Takata +. He died April 25, 1940, 2½ years after entry. Portal thrombosis was suspected. There was no autopsy.

*Case 29.* A 53-year-old American janitor entered the hospital December 21, 1937, because of abdominal swelling. He gave a history of alcoholism and a diet poor in meat and vegetables. His Wassermann had been positive in 1931. He had had arsphenamine therapy in 1931 and 1932. He had had paresthesias in legs for several years, intermittent swelling of legs and abdomen for one year and persistent swelling for 2 months. He was hospitalized on November 29, 1937 and had an abdominal tap. His weight fell from 174 to 148 lbs. He received diuretics and minimal vitamin therapy. Ascites recurred in 3 weeks and he was transferred to Research Service. Chief findings were: Dry, rough skin, polyneuritis, glossitis, vascular "spiders", palpable liver and spleen, ascites, collateral veins; RBC 3.8, Hgb. 75%, WBC 8,000, serum albumin 2.5, globulin 3.4, bromsulfalein 28%, Takata +, icteric index 5.

He was given intensive vitamin therapy at entry and had diuresis in 5 days. His weight fell from 165 to 130 lbs., with loss of ascites and edema. Albumin rose from 2.5 to 3.2 in 10 days. For the next 2 years the patient was ambulatory and well. His weight rose from 130 to 150 lbs. He had no G.I. disturbances; no ascites or edema. Laboratory data, however, remained un-

changed: Serum albumin 3.1 to 3.5, icteric index 5 to 12, bromsulfalein 15 to 40%, Takata 2+ to 4+, globulin 3.5 to 4.0. He was discharged February 6, 1940, and returned 1 month later with massive ascites. He was mentally confused (interval history unreliable). Laboratory data were: Serum albumin 2.4, globulin 3.4, RBC 4.7, Hgb. 90%, WBC 13,500. There was rapid onset of coma, with death 4 days after entry. Autopsy showed portal cirrhosis of liver.

*Case 28.* An 83-year-old Italian laborer entered the hospital September 16, 1937, for abdominal swelling. He gave a history of alcoholism, syphilis and poor diet (0 meat, 0 vegetables). He had had a course of arsphenamine 5 years previously. Anorexia and abdominal swelling had been present for 1 year. He entered the hospital in January 1937. Chief findings were: Ascites, edema, hydrothorax; icteric index 18, bromsulfalein 50%, Wassermann negative. He received three abdominal taps and mercurial diuretics; was transferred to Research Service September 16, 1937. Chief findings were: Emaciated old man; dry, rough skin, pigmentation, glossitis, collateral veins, ascites, edema, B.P. 135/75; RBC 3.7, Hgb. 75%, WBC 7,800, serum albumin 1.8, globulin 5.3, Takata +, icteric index 9, bromsulfalein > 40; Wassermann negative. There was a question of peripheral neuritis. Liver and spleen were not felt.

He had two abdominal taps in 2 months. Mercupurin injections at intervals controlled minimal ascites and edema for 8 months. After 1 year no ascites or edema was present. Serum albumin in 1 year rose from 1.8 to 3.0; globulin varied from 4.6 to 5.6; Takata remained +; icteric index rose from 5 to 10; bromsulfalein was ± 40. Weight was between 120 and 125 lbs. His course was stationary throughout the second year. There was no ascites. The patient was ambulatory. Laboratory values remained unchanged. In September 1939, 2 years after entry, there was increasing jaundice. The icteric index rose gradually to 50 in November; albumin fell from 3.0 to 2.1. Hgb. fell from 80 to 67%. The patient lapsed into coma and died on January 23, 1940, 2 years and 4 months after entry. Autopsy showed carcinoma of caudate lobe, cirrhosis (portal) of liver. The terminal 4-month course was probably due to the superimposed carcinoma.

*Case 35.* A 38-year-old Porto Rican entered the hospital November 15, 1938, because of swollen abdomen. She gave a history of alcoholism and poor diet (0 meat; 0 vegetables). She had had rheumatic fever 10 years previously and a hysterectomy 5 years previously. She had had recurrent epistaxes for 4 years, occasional tarry stools for 3 years, purpuric spots and easy bruising for 2 years, intermittent jaundice for 2 years. She had lost 17 lbs. in 1 year, had had paresthesias for 1 year and swelling of abdomen and ankles for 8 months. She entered another hospital 2 months before being transferred to Research Service. Chief findings were: Jaundice, ascites, edema, palpable liver and spleen, collateral veins, vascular "spiders", mitral valvulitis; RBC 2.2, Hgb. 48%, WBC 5,300, platelets 80,000, icteric index 20, cholesterol 485, Takata +, trace of bile in urine.

Stools were negative for schistosoma. She received a high CHO diet and vitamin supplements. When transferred to Research Service, findings were as above. In addition, glossitis, mild peripheral neuritis, nystagmus and pigmentation over tibiae were present. On November 7, 1938, laboratory data were: RBC 2.34, Hgb. 59%, WBC 8,000, Wassermann 2+, albumin 2.9, globulin 4.5, Takata +, icteric index 50, bromsulfalein 80%, trace of bile in urine.

Ascites increased in the first 2 weeks after hemorrhage from vascular "spider" on lip; bleeding point was cauterized; patient was transfused with 400 cc. blood. Diuresis was followed by a 20 lb. weight loss and disappearance of ascites and edema during the next 2 months. The patient showed a marked gain in strength, felt well and left against advice. On discharge, 4 months after entry, laboratory data were: RBC 3.0, Hgb. 69%, WBC 6,800, icteric index 20, albumin 3.4, globulin 4.1, Takata +, bromsulfalein 70%. She entered another hospital 1 month later because of massive hematemesis and was transfused. Liver biopsy showed portal cirrhosis. She was discharged after 1 month and seen at home July 9, 1939, when she was ambulatory but weak. She had slight jaundice, albumin 3.7 and globulin 4.6. There was no ascites. She returned to Puerto Rico and there was no follow up.

*Case 39.* A 58-year-old Swedish watchmaker entered the hospital May 24, 1939, because of swollen abdomen. He gave a history of alcoholism, inadequate diet, occasional diarrhea and cramps in fingers and toes. He had had daily epistaxes for 6 months and ascites for 1 month. He was hospitalized May 4, 1939. There was partial loss of ascites with mercurial diuretics and minimal vitamin therapy. His weight fell from 176 to 165. He was transferred to Research Service 3 weeks later. Chief findings were: dry, rough skin, jaundice, vascular "spiders", pigmentation, collateral veins, palpable liver and spleen, ascites, edema, peripheral neuritis; RBC 3.1, Hgb. 65%, WBC 7,600, icteric index 25, bromsulfalein 86%, albumin 2.9, globulin 5.7, Takata +.

Diuresis continued after entry. He received a nutritious diet without vitamin supplements and felt moderately well. His icteric index fell to 8; bromsulfalein to 68%. Blood counts and serum proteins remained unchanged. He was discharged August 12, 1939, and followed in O.P.D. Although there was no recurrence of ascites, the patient felt poorly and complained of fatigue. Mild icterus was consistently present (icteric index 20 to 40). He was re-admitted on December 18, 1939 and a nutritious diet and vitamin B concentrates were given. His weight increased 20 lbs. in 4 months. There was no ascites. Polyneuritis and jaundice subsided. He was discharged on May 24, 1940, and followed in O.P.D. On November 16, 1940, laboratory data were: albumin 4.0, globulin 4.3, icteric index 12, bromsulfalein 72%. The patient felt much improved 1½ years after first entry.

*Case 40.* A 50-year-old Greek clerk entered the hospital January 12, 1939, because of abdominal swelling. He gave a history of alcoholism. His diet had been

adequate. He had no history of malaria. Three years previously (November, 1935) he had had swelling of abdomen and legs and had been hospitalized for 7 weeks. One abdominal tap had been performed. He was readmitted 4 times at monthly intervals for abdominal taps. Since July, 1936, the ascites had been partially controlled by weekly injections of mercurial diuretics and daily ingestion of saline diuretics. He was never ascites-free. One year before admission to Research Service he had had a transient hemiplegia. For 1 year he had had paresthesias in legs. He reentered the hospital because of failure of the ascites to respond to diuretics and was transferred to Research Service January 12, 1939. Chief findings were: hepatic facies, vascular "spiders", increased pigmentation, B.P. 180/110, umbilical and inguinal herniae, collateral veins, ascites, palpable liver and spleen, hemorrhoids, edema; venous pressure 94, circulation time 14 seconds, RBC 4.2, Hgb. 92%, WBC 4,500, albumin, RBC's and casts in urine, bromsulfalein 30%, Takata +, serum albumin 2.7, globulin 3.3, icteric index 3. There was no cardiac enlargement.

One abdominal tap was performed 2 weeks after entry. Saline diuretics were given for 6 months. There was a gradual disappearance of ascites and edema and the patient was free of ascites and edema for 1 year. Liver and spleen were barely palpable. Vascular "spiders" disappeared. Hypertension and urinary changes persisted. Pyelogram showed destructive lesion in the kidney pelvis. Guinea pig showed + Tbc. He was discharged against advice on July 1, 1940, 1½ years after entry. Laboratory data were: RBC 4.2, Hgb. 88%, WBC 5,200, bromsulfalein 14%, icteric index 5, serum albumin 3.0, globulin 2.8, Takata negative. The patient was ambulatory but not strong. When he was seen 2 months later, the ascites was returning.

*Case 32.* A 45-year-old Greek waiter entered the hospital March 13, 1940, because of hematemesis. He had had three courses of arsphenamine therapy 15 years previously and 3 injections yearly thereafter. His diet had been poor in meats and fats. He did not take alcohol. He had had G.I. distress for many years and had taken cathartics daily for 5 years for constipation and hemorrhoids. For 1½ years he had had severe epigastric pain 1 to 2 hours after meals. Tarry stools had been noted. In February, 1940, he had had two massive hematemeses followed by onset of ascites. He was hospitalized. Fluoroscopy showed esophageal varices. He was transferred 1 month later to Research Service. Chief findings were: Pallor, vascular "spiders", palpable liver and spleen, ascites, collateral veins; RBC 3.34, Hgb. 53%, WBC 3,450, Takata +, bromsulfalein 30%, serum albumin 3.2, globulin 3.1, icteric index 7. There was no jaundice.

Two months after entry he had a sudden melena for which there was no apparent cause. After that, the patient made steady gain in weight and strength. Ascites disappeared. He was discharged September 5, 1940 and resumed work. On discharge laboratory data were: RBC 4.2, Hgb. 93%, bromsulfalein 24%, icteric index 7, serum albumin 3.8, globulin 3.7. He was seen January

11, 1941, 10 months after entry. There was no change. He felt well.

*Case 33.* A 46-year-old American waitress entered the hospital February 26, 1940, because of abdominal swelling for 3 months. An accurate history was not obtainable. She had had a salpingectomy 15 years previously and pneumonia 4 years previously. She gave a history of chronic alcoholism and poor diet (no meat or dairy foods). She was admitted with delirium tremens. Chief findings were: disoriented, confused, undernourished woman; dermatitis, vascular "spiders", jaundice, glossitis, palpable liver, ascites, collateral veins, pigmented legs, edema, polyneuritis, perineal abscess; RBC 3.3, Hgb. 65%, WBC 15,000, albumin 2.3, globulin 3.5, icteric index 15, bromsulfalein 64%, Takata +, urine negative.

The patient was in a critical condition for 6 weeks with a temperature of 100° to 102° F. She was confused, incontinent of urine and feces. One abdominal tap was performed. Diuresis with loss of ascites and edema occurred after 8 weeks. Thereafter, she showed striking general improvement: incontinence subsided; she became mentally lucid; gained 20 lbs.; dermatitis, glossitis, and polyneuritis subsided; vascular "spiders" and jaundice disappeared; liver edge receded; spleen was not palpable. On discharge August 15, 1940, 6 months after entry, laboratory data were: RBC 4.3, Hgb. 75%, WBC 8,000, icteric index 5, bromsulfalein 8%, Takata negative, serum albumin 4.8, globulin 3.2. The patient was seen in O.P.D. 2 months later and there was no clinical change; she felt well.

*Case 34.* A 50-year-old American housewife entered the hospital March 19, 1940, because of abdominal swelling. She gave a history of chronic alcoholism and poor diet (no meat, no dairy foods). She had had repeated nose bleeds for 5 years and intermittent abdominal distress for 4 years; she had known of her hypertension for 2 years. In the year before her admission to the hospital her weight had fallen from 145 to 121. *Five months before* (October 1939) she had been hospitalized for 1 month. Chief findings were: delirium tremens, jaundice, moderate ascites, edema, palpable liver and spleen, collateral veins; icteric index 20, Kline 2+, B.P. 166/88. *One month before* she had been hospitalized with similar findings. A peritoneoscopy, with removal of about 1 liter of fluid, showed "hob-nail" liver. She received injections of thiamin chloride and ascorbic acid, glucose infusions and mercurial diuretics. She was transferred to Research Service March 19, 1940. Chief findings were: Pale, nervous, irritable woman; mentally cloudy, coarse tremor of hands, lateral nystagmus, many vascular "spiders" on face and thorax, subicteric sclerae, glossitis, bleeding gums, palpable liver and spleen, collateral veins, moderate ascites. B.P. varied from 130/80 to 190/100; venous pressure 85, circulation time 9 seconds. There was no peripheral edema and no cardiac enlargement. Laboratory data were: RBC 3.5, Hgb. 63%, WBC 8,500, albumin 2.8, globulin 4.0, icteric index 15, Takata +, bromsulfalein 68%.

The patient showed a rapid gain in strength and weight, with loss of tremor. Ascites disappeared in 2

months. She became mentally clear and was discharged on June 29, 1940, 3 months after entry. Laboratory data were: RBC 4.4, Hgb. 89%, WBC 8,700, albumin 3.9, globulin 4.2, Takata +, icteric index 5, bromsulfalein 50%. She was seen in O.P.D. 6 months later and felt entirely well; there was no clinical change.

*Case 36.* A 40-year-old Porto Rican entered the hospital May 1, 1940, because of jaundice and G.I. distress. He gave a history of alcoholism and poor diet. He had had migratory polyarthritides for 15 years, epistaxes for 10 years, anorexia, nausea and vomiting for 2 years. He had had jaundice for 2 months, followed by onset of ascites. He was hospitalized September 6, 1939, and received mercupurin injections and vitamin supplements. Diuresis with loss of ascites occurred in 5 weeks. His weight 10 years previously had been 172; 1 year previously 155; before diuresis 140; after diuresis 120 lbs. He was discharged October 23, 1939, and was seen in O.P.D. 1 week later. Chief findings were: Vascular "spiders", pigmentation, slight jaundice, glossitis, collateral veins, palpable liver, polyneuritis; RBC 3.5, Hgb. 78%, WBC 4,100, albumin 3.9, globulin 4.6, icteric index 12.5, Takata +, bromsulfalein 40%. There was no splenomegaly and ascites was doubtful.

The patient was seen periodically in O.P.D. and was maintained on vitamin therapy. He gained 30 lbs. in 7 months and gained in strength. There was no ascites. He reentered the hospital May 1, 1940, with fever, nausea, vomiting, and diarrhea of 3 weeks' duration. In addition to previous findings, he had jaundice (icteric index 25) and a palpable spleen. His temperature was 100 to 102° F. for 5 days. The jaundice cleared. He was discharged in 3 weeks. There was no essential change in blood counts or proteins. He resumed work May 20, 1940. On October 1, 1940, 1 year after his first hospital entry, there was no clinical change.

### Group 3

*Case 8.* A 48-year-old Italian butcher entered the hospital September 16, 1937, because of abdominal swelling. He gave a history of chronic alcoholism and poor diet. He had had arsphenamine therapy 3 years previously and nausea and vomiting for 1 year. He had had swelling of abdomen, legs and scrotum for 5 months, with occasional diarrhea and melena. Three abdominal taps had been performed the previous month in another hospital from which he was transferred to Research Service. Chief findings were: Emaciation, glossitis, right hydrothorax, ascites, edema, palpable liver and spleen, vascular "spiders", RBC 4.0, Hgb. 68%, WBC 3,000, serum albumin 2.4, globulin 2.7, Takata +, Kahn +, bromsulfalein 40%, icteric index 4, venous pressure 65 mm.

Two abdominal taps were performed in the first 2 weeks. The patient failed rapidly; became incontinent of urine and feces and ran a terminal fever (Temperature 102° F.). He died October 11, 1937, 1 month after entry. Autopsy showed portal cirrhosis; purulent pericarditis (pneumococcus type 6); phlegmonous enteritis and colitis.

*Case 13.* A 55-year-old American housewife entered the hospital April 3, 1937, because of abdominal swelling. She gave a history of chronic alcoholism and poor diet. She had had nausea, vomiting and weakness for six months, with occasional diarrhea. Four months before admission she developed jaundice, followed by swelling of abdomen and legs, and increasing mental confusion. She entered another hospital and was transferred to Research Service after three abdominal taps had been performed. Chief findings were: Emaciated, euphoric woman; lateral nystagmus, atrophy of tongue, ascites, dry skin, edema, wrist and foot drop, absent knee and ankle jerks, palpable spleen; RBC 3.3, Hgb. 61%, WBC 10,000, serum albumin 2.2, globulin 3.2, bromsulphalein 40%, Takata +, icteric index 3. Liver could not be felt.

There was progressive failure, with abdominal taps at biweekly intervals. The patient died June 14, 1937, 2 months after entry. Autopsy showed portal cirrhosis of liver.

*Case 11.* A 58-year-old Irish longshoreman entered the hospital because of abdominal swelling. He had had arsphenamine therapy 27 years previously; malaria 25 years previously; was a chronic alcoholic; and his diet was inadequate. His best weight had been 208. His weight on admission was 144 lbs. He had had rheumatic fever 2 years previously with carditis (fibrillation); ascites had been noted 1 year previously. One abdominal tap had been performed at another hospital. Thereafter, biweekly diuretics controlled ascites until 1 month before admission. He was transferred to Research Service. Chief findings were: Emaciated, elderly man; skin dry and scaly; legs pigmented and purpuric; teeth missing; heart slightly enlarged, slow fibrillation, rate 75 per minute; B.P. 130/75; ascites; umbilical hernia; collateral veins prominent; hemorrhoids. Liver and spleen could not be felt; no CNS changes. Laboratory data were: RBC 3.9, Hgb. 81%, WBC 6,200, venous pressure 98, circulation time 15 seconds, bromsulphalein 40%, Takata +, icteric index 5, serum albumin 2.2, globulin 4.5, total cholesterol 238, ester 152. E.K.G. showed auricular fibrillation.

The patient failed rapidly. Three abdominal taps were performed. He died in coma, 1 month after entry. Autopsy showed portal cirrhosis, bilateral obliterative pleuritis, obliterative pericarditis, fibrous encapsulation of liver and spleen, chronic pancreatitis.

*Case 19.* A 57-year-old Italian laborer entered the hospital March 28, 1938, because of abdominal swelling. There was no history of jaundice or alcoholism. He had had a rapid onset of ascites 7 months previously and cramp-like lower abdominal pains for 5 months. He had had a laparotomy 2 months before admission. A "small, shrunken, nodular liver" had been found. His usual weight had been 165; his weight on admission was 134 lbs. Chief findings were: Emaciated, pale man; skin dry and scaly; heart and lungs normal; B.P. 115/90; prominent abdominal veins; ascites; bilateral inguinal herniae; hernia of operative wound. No vascular "spiders", liver and spleen not felt. Laboratory data were:

RBC 5.2, Hgb. 92%, WBC 6,000, serum albumin 3.6, globulin 4.3, Takata +, bromsulphalein 28%, icteric index 7, cholesterol 207.

The patient had abdominal taps every 3 weeks. His course was stationary until he had a massive hematemesis on September 9, 1938. He responded well to transfusion. Signs of pneumonia appeared on January 13, 1939, when he had a white cell count of 34,000. Death occurred 4 days later. Autopsy showed portal cirrhosis, esophageal varices, lobular pneumonia; organized, old portal thrombosis, fresh retrograde thrombosis of mesenteric veins.

*Case 21.* A 48-year-old German chef entered the hospital October 22, 1937, because of swollen abdomen. He was a chronic alcoholic. He had had salvarsan and bismuth injections 9 years previously. He had been hospitalized 2 years ago (1935) because of abdominal pain and vomiting; a large liver had been noted. He was placed on a high CHO, low protein, low fat diet; the symptoms persisted. He had occasional diarrhea. He entered another hospital 1 month previously and was transferred to Research Service after one abdominal tap. His usual weight was 175; his weight on admission was 155. Chief findings were: Pallor, vascular "spiders", ascites, edema, collateral veins, left hydrothorax, B.P. 145/88, liver and spleen palpable. There were friction rubs over liver and spleen. The heart was not enlarged. There were no CNS changes. Laboratory data were: RBC 2.27, Hgb. 55%, hematocrit 26, WBC 3,000, serum albumin 2.9, globulin 3.4, icteric index 5, Takata +, bromsulphalein 36%, Kahn 2+, venous pressure 30, circulation time 10 seconds. E.K.G.'s were normal. The ascitic fluid contained 3% albumin.

The patient ran a spiking temperature from 98 to 101° F. daily throughout his 14-month course. Cultures of blood and pleural and ascitic fluids were sterile. Abdominal taps were performed at 3-week intervals for 3 months. Two transfusions of 250 cc. each were given. He had no taps for 3 months. Moderate diuresis occurred with loss of ascites. He gained in strength. Serum albumin rose from 2.2 to 3.5 grams. Thereafter, there was progressive failure for 8 months. He had recurrent ascites with weekly paracenteses; the ascitic fluid became bloody; epistaxes and hematuria appeared; there was no response to vitamin K therapy; an abdominal tap performed December 18, 1939, was grossly bloody. On December 19, 1939, laboratory data were: RBC 2.0, Hgb. 37%, WBC 32,000, serum albumin 2.0, globulin 2.3, Takata +, bromsulphalein > 40, icteric index 5. The patient died in shock despite a transfusion of 500 cc. blood. Autopsy showed portal cirrhosis, esophageal varices, hemoperitoneum, fibrous adhesions of pericardium, pleura, and peritoneum; encapsulation of liver and spleen by dense fibrous tissue.

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# THE INTRAVENOUS GLUCOSE TOLERANCE TEST

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Oral glucose tolerance tests have generally been used to differentiate between diabetes mellitus and the various forms of non-diabetic glycosuria (1). On the basis of such oral tests alterations in carbohydrate metabolism have also been reported in numerous disorders other than diabetes mellitus. These include endocrine, intracranial and hepatic diseases (2), arthritis (3), and convulsive disorders of various types (4). Oral tests, however, necessarily fail to distinguish between effects due to changes in intestinal absorption and those due to alterations in carbohydrate metabolism. Variations in gastric emptying time and in intestinal absorption are in fact known to influence the results of oral tests (5). An intravenous glucose tolerance test automatically eliminates variations due to gastro-intestinal factors. The present study describes the results obtained with a standard intravenous glucose tolerance test. The extent of variation of the test was first determined in normal individuals. It was then applied to patients with a variety of conditions in which the carbohydrate metabolism might possibly be deranged.

## METHODS

Sixty normal adult subjects were studied. All were in the post-absorptive state and remained in a recumbent position throughout the morning. After a fasting venous blood sample had been obtained, 50 ml. of a 50 per cent solution of glucose in distilled water were injected intravenously during a period of two minutes. Samples of venous blood were obtained at intervals during the next two or three hours. In the majority of cases hourly urine samples were collected as well. A modification of Benedict's macro-method (6) was used for blood sugar analyses, protein-free filtrates of whole blood being employed. Protein was removed by the zinc precipitation method of Somogyi (7), which also removes most of the non-fermentable reducing substances. Rothberg and Evans' (8) tubes were used in the final visual colorimetric comparison. Urine samples were analyzed for glucose by the quantitative method of Benedict (9).

The same procedure was applied to 271 patients in the

New Haven Hospital. Only a few with outright diabetes were included. The majority were selected either because, on clinical grounds, some abnormality of carbohydrate metabolism was suspected or because they had some one of a variety of disorders reputedly attended by derangement of carbohydrate metabolism.

## RESULTS

### *(A) Analysis of results in sixty normal subjects*

The original data are presented in Table I. The form of the curve derived from these data relating blood glucose values to time is indicated in Figure 1. The heavy line passes through the mean values, while the lighter upper and lower curves represent the extreme limits of variation. The blood glucose rises sharply immediately following the intravenous injection of glucose, then begins to fall with almost equal rapidity. The rate of fall is, however, progressively retarded during the next two hours. The rate of fall of the blood glucose varied considerably. In some of the subjects the blood glucose fell to the fasting value within half an hour, while in others it was still elevated after an hour and a half. In all subjects, by two hours the blood sugar was within the normal fasting range.

In every instance the blood glucose fell below the initial fasting concentration at some time within the first two hours. This feature of the individual curves of Table I is lost when the average curve of Figure 1 is drawn. In many instances the blood sugar subsequently rose above the minimum point. The low point was reached at forty-five minutes in four tests; at sixty minutes in eight tests; at ninety minutes in thirteen tests and at two hours in thirty-one more. In four of the ten curves in which the blood sugar was determined after three hours, the concentration was still lower than it had been after two hours.

Hypoglycemia therefore regularly occurs in the normal individual following the intravenous in-

TABLE I  
*Raw data of intravenous glucose tolerance tests on sixty normal subjects*

Number	Age	Sex	Height	Weight	Blood glucose level at minutes indicated after intravenous injection of 25 grams of glucose									Urinary excretion of glucose at hours indicated after injection			
					0'	5'	15'	30'	45'	60'	90'	120'	180'	1	2	3	Total
	years		cm.	kgm.	milligrams glucose per 100 ml. blood									grams			
1	25	F	163	63.5	94	222	167	87	78	78		87					
2	39	M	173	59.0	92	286	182	131	90	74		68		1.17	0		1.17
3	29	F	165	51.5	100	304	213	177	142	126		78		1.95	0.04		1.99
4	43	F	168	68.0	110	273	204	153	129	101		77		1.59	0.66		2.25
5	52	M	160	63.0	82	254	208	145	127	99		67		1.13	0.08		1.21
6	22	F	168	53.5	82	296	234	191	153	102		87		0.83	0		0.83
7	24	M	173	62.5	92	263	191	141	95	76		78		1.82	0		1.82
8	21	F	180	69.0	91	206	177	112	68	65		82		0.88	0		0.88
9	25	F	173	57.5	98	282	203	154	110	100		85		1.27	0.35		1.62
10	26	M	185	90.5	86	263	144	88	76	67		89		0.93	0		0.93
11	27	M	180	81.5	90	255	200	175	146	120		83		1.06	0.09		1.15
12	26	F	160	49.0	87	286	195	139	110	100		80					
13	22	F	156	51.0	92	328	229	174	97	69		78		2.06	0.06		2.12
14	34	F	160	54.5	84	291	193	119	90	60		67		1.83	0		1.83
15	33	F	160	50.0	87	271	164	76	53	58		80		1.66	0		1.66
16	30	M	188	81.5	80			110	92	86	82	90		0.73	0		0.73
17	28	M	173	70.0	86			98	67	68	86	87		1.31	0		1.31
18	41	M	170	76.5	110			207	165	147	107	84		0.59	0		0.59
19	49	M	160	60.5	79			125	108	78	67	71		0.89	0		0.89
20	65	M	173	57.0	89			165		136	107	90					
21	44	M	168	60.5	82			143		99	89	82					
22	40	M	173	60.5	84			137		90	68	67					
23	47	M	165	80.0	92			131		102	91	90					
24	40	M	168	54.0	86			171		135	95	79					
25	35	M	173	64.0	79			157		138	106	89					
26	49	M	183	96.0	75			96	77	71	82	85		0.51	0		0.51
27	35	M	173	64.5	117			74	60	66	67	83					
28	29	M	180	64.5	79			126	93	78	57	67		0.83	0		0.83
29	37	M	152	49.5	82			135	95	79	76	78					
30	26	M	157	55.5	85			128	89	76	78	85					
31	52	M	165	79.0	91			158	135	117	96	89					
32	74	M	168	59.0	90			130	111	102	78	80		0.36	0.11		0.47
33	35	M	157	52.0	75			141	109	90	70	65		1.43	0		1.43
34	53	M	180	87.5	105			180	135	129	103	84		0.77	0.12		0.89
35	54	M	160	67.0	82			131	112	99	79	68		1.31	0		1.31
36	53	F	165	105.0	85			121	105	95	83	82		0.5	0		0.5
37	60	M	165	54.5	79			166	143	122	85	75		0.79	0.04		0.83
38	49	M	178	90.5	78			115	84	72	71	76		0.47	0.09		0.56
39	39	F	160	74.5	90			163	120	110	85	77		0.92	0.07		0.99
40	21	F	160	55.5	99			215		151	85	74		pooled			1.48
41	46	F	168	76.5	85			149	109	84	68	67					
42	49	F	165	84.0	92			133	117	97	86	89		0.64	0		0.64
43	24	F	157	53.0	85			126	94	86	82	85		1.13	0		1.13
44	42	M	183	78.5	95			163	150	117	77	71					
45	49	M	185	82.0	93			106	88	71	70	85		0.68	0		0.68
46	39	M	163	60.5	82			152	131	112	67	66		pooled			1.16
47	23	M	170	68.0	89			108	77	76	85	86		1.29	0		1.29
48	48	M	157	71.0	100			162	115	93	78	78		1.39	0		1.39
49	24	M	175	63.5	84			79	68	71	81	84		0.58	0		0.58
50	59	M	185	93.0	88			136	105	105	84	75		0.90	0		0.90
51	58	M	170	73.5	82					127	96	82	81	0.27	0		0.27
52	40	M	170	54.5	101					114	82	72	79	1.68	0	0	1.68
53	50	M	163	78.5	72					115	93	74	67	1.05	0.10	0.07	1.22
54	52	M	165	77.5	100					131	96	75	76	0.71	0	0	0.71
55	43	M	165	57.0	87			168		120	89	77	72	0.30	0	0	0.30
56	44	M	165	71.5	85			136		78	75	81	81	0.51	0	0	0.51
57	52	M	170	78.0	85					80	75	78	81				
58	44	M	175	57.0	96					116	79	75	76	0.93	0	0	0.93
59	26	M	168	61.0	108					111	86	84	71	1.50	0	0	1.50
60	56	M	168	67.0	88					94	76	67	71	1.21	0.36	0.08	1.65

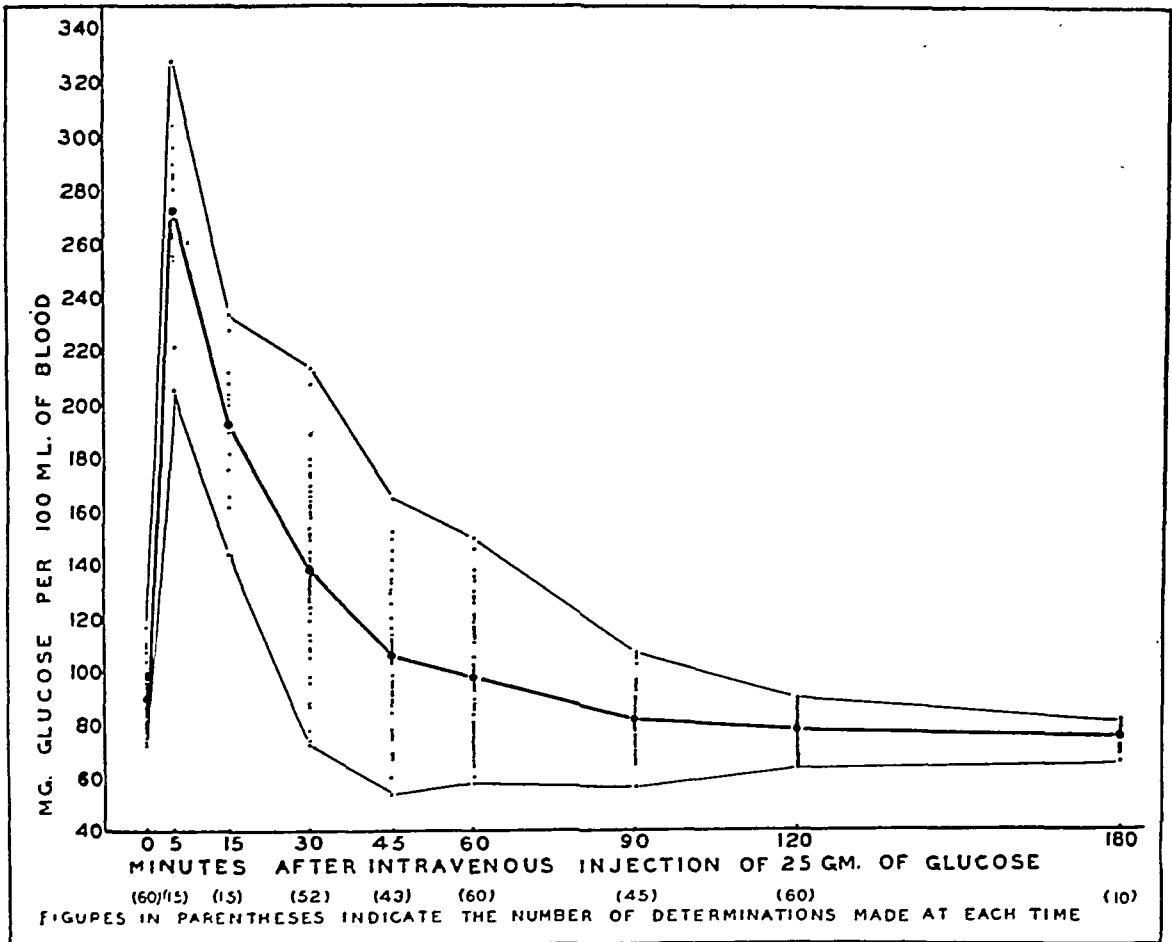


FIG. 1. RESULTS OF INTRAVENOUS GLUCOSE TOLERANCE TESTS ON SIXTY NORMAL SUBJECTS

Upper and lower light solid lines indicate maxima and minima, respectively. Heavy solid line connects arithmetical means.

jection of glucose. The extent of this hypoglycemic response could not be correlated with the fasting blood sugar. Hypoglycemia was as apt to appear promptly in individuals with high initial blood sugar concentrations as in those with low initial concentrations. Indeed the initial blood sugar could not be correlated with any characteristic of the curve.

For statistical purposes the standard deviation of the mean at each point was computed by the formula,

$$\text{S.D.} = \sqrt{\frac{\sum d^2}{N-1}},$$

$\sum d^2$  representing the sum of the squares of the individual deviations and  $N$  the number of observations at each point. The mean, the standard

deviation of the mean, and the maximum and minimum values at each point are presented in Table II. The standard deviation is comparatively

TABLE II

*Analysis of glucose tolerance tests on sixty normal subjects*

Minutes after injection	Number of subjects	Mgm. glucose per 100 ml. blood			
		Maximum	Minimum	Arithmetical mean	Standard deviation
0	60	117	72	89.0	9.1
5	15	328	206	272.0	30.7
15	15	234	144	193.3	24.3
30	52	215	74	138.7	31.8
45	43	165	53	105.6	26.7
60	60	151	58	97.1	23.5
90	45	107	57	82.7	11.5
120	60	90	65	78.8	7.2
180	10	81	67	75.5	5.0

large at the earlier points but becomes progressively smaller, so that by two hours it has become less than it was at the initial observation. The still lower standard deviation at three hours has less significance since data from only ten subjects are available.

Glycosuria, which was absent in only one subject, was usually confined to the first hour, but in three of the subjects it continued during the second hour as well. The maximum amount excreted by any normal subject was 2.25 grams, less than 10 per cent of the amount injected.

The justification of the use of a fixed dose of 25 grams, rather than a dose adjusted to the size of the subject, appears from an examination of

Table I. The subjects ranged in weight from 45 to 105 kilograms, in age from 21 to 74 years, and in height from 152 to 188 centimeters. About one quarter of the subjects were females. Nevertheless, in spite of this variation in the physical characteristics of the subjects, no correlation between the results of the tolerance test and sex, age, height or weight could be demonstrated. Adjustment of the dose to weight or height, therefore, is without logical basis.

(B) *Analysis of results in 271 patients*

The curves of patients resembled in general characteristics those of normal subjects. Not infrequently the blood sugar fell more slowly than

TABLE III  
*Analysis of blood glucose levels at 120 minutes after intravenous injection of 25 grams of glucose in 271 patients*

Clinical diagnosis	Number of cases	Distribution			
		Normal	Moderately elevated	Markedly elevated	Moderately depressed
		65-99 mgm. per 100 ml.	100-119 mgm. per 100 ml.	120-281 mgm. per 100 ml.	38-64 mgm. per 100 ml.
Tests done because of glycosuria					
(a) Definite diabetes mellitus.....	19	3	6	10	0
(b) Probable diabetes mellitus.....	14	1	1	12	0
(c) Possible diabetes mellitus.....	8	2	2	4	0
(d) No evidence of diabetes mellitus.....	78	67	6	3	2
Tests done because of pituitary disease					
(a) Acromegaly (without glycosuria).....	6	4	2	0	0
(b) Verified chromophobe adenoma.....	3	3	0	0	0
(c) Unverified chromophobe adenoma.....	2	2	0	0	0
(d) Typical basophilism.....	7	5	2	0	0
(e) Atypical basophilism (obesity, hirsutism).....	29	27	2	0	0
Tests done because of other endocrine diseases					
(a) Typical hyperthyroidism.....	4	3	1	0	0
(b) Atypical hyperthyroidism.....	3	2	1	0	0
(c) Addison's disease.....	1	1	0	0	0
(d) Myxedema.....	1	1	0	0	0
Tests done for suspicion of hypoglycemia					
(a) Verified islet cell adenoma of pancreas.....	2	1	0	0	1
(b) Unverified islet cell adenoma of pancreas.....	1	1	0	0	0
(c) Idiopathic epilepsy.....	16	14	0	0	2
(d) Convulsions without true epilepsy.....	3	3	0	0	0
(e) Syncope with vasomotor disturbances.....	16	13	2	0	1
(f) Syncope without vasomotor disturbances.....	18	18	0	0	0
Tests done because of pancreatic disease					
(a) Steatorrhea.....	3	1	0	0	2
(b) Acute and chronic pancreatitis.....	3	2	1	0	0
Miscellaneous tests					
(a) Anorexia nervosa and marked malnutrition.....	5	2	1	2	0
(b) Verified brain tumor.....	4	3	0	0	1
(c) Unverified brain tumor.....	4	3	1	0	0
(d) Liver disease.....	7	3	3	0	1
(e) Arthritis.....	5	3	2	0	0
(f) Various specific diseases.....	5	4	1	0	0
(g) No organic disease.....	4	4	0	0	0
Totals.....	271	196	34	31	10

TABLE IV

*Analysis of blood glucose levels 120 minutes after intravenous injection of 25 grams of glucose in 119 patients with glycosuria, with respect to criteria for diagnosis of diabetes mellitus*

Evidence for diagnosis of diabetes mellitus	Number of cases	Distribution			
		Normal	Moderately elevated	Markedly elevated	Moderately depressed
		65-99 mgm. per 100 ml.	100-119 mgm. per 100 ml.	120-281 mgm. per 100 ml.	88-64 mgm. per 100 ml.
Definite evidence (19 Cases)					
(a) History of treatment with insulin in amounts greater than 30 units per day, usually associated with marked hyperglycemia.....	14	2	5	7	0
(b) History of fasting hyperglycemia greater than 200 mgm. per 100 ml.....	5	1	1	3	0
Probable evidence (15 Cases)					
(a) History of fasting hyperglycemia between 150 and 199 mgm. per 100 ml.....	12	1	2	9	0
(b) History of fasting hyperglycemia between 120 and 149 mgm. per 100 ml. together with glycosuria, polyuria and polydipsia.....	3	0	0	3	0
Possible evidence (7 Cases)					
(a) History of glycosuria, polyuria and polydipsia without history of fasting hyperglycemia.....	2	0	1	1	0
(b) History of fasting hyperglycemia between 120 and 149 mgm. per 100 ml. without symptoms.....	5	2	0	3	0
No evidence other than glycosuria.....	78	67	6	3	2
Grand totals.....	119	73	15	29	2

did that of normal subjects; only rarely did it fall more rapidly. Deviations from normal were most evident at the two-hour point, since here the range of normal variation was so small. In statistical phraseology, the standard deviation of the mean reached its minimum significant value at this point (Table II). Any individual value differing from the mean normal value by an amount greater than three times the standard deviation of this mean was considered outside the normal range. In any series having a normal frequency distribution, only one in a hundred of the individual values will differ from the arithmetical mean by an amount exceeding three times the standard deviation.

The patients were therefore divided into four groups on the basis of the blood sugar concentration two hours after the injection of glucose. These are: (a) a group with blood glucose concentrations within the normal range, from 65 to 99 mgm. per 100 ml.; (b) a group with moderate elevation of the blood sugar, ranging between 100 and 119 mgm. per 100 ml.; (c) a group with markedly elevated blood sugar values, ranging between 120 and 281 mgm. per 100 ml.; and (d) a group with blood glucose below the lowest con-

centration in the normal group, *i.e.*, less than 65 mgm. per 100 ml. Values in group (a) differed from the normal mean by less than three times its standard deviation; those in group (b) by more than three but less than six times; and those in (c) by more than six times. Of the 271 patients, 196 fell into class (a), thirty-four into class (b), thirty-one into class (c) and ten into class (d). After the patients had been divided into these four groups on the basis of the glucose tolerance test alone, their clinical records were consulted. Diagnoses were then assigned to each patient, using all available clinical and pathological criteria *except* the results of the glucose tolerance test itself. The results of this analysis are presented in Table III. The group of patients in whom the test was carried out because of glycosuria was further analyzed with respect to criteria used in making or excluding the diagnosis of diabetes mellitus. The results are presented in Table IV. The small proportion of cases with outright diabetes is due to the fact that the test was usually not done in such cases. Borderline cases form an unusually high percentage of the entire group, since such cases were deliberately selected. Had the tests been done rou-

tinely on all glycosuric patients, a very much larger proportion of cases with clear-cut diabetes would have been included.

#### DISCUSSION

The type of test used in this study is similar to the usual type of oral test, in that its interpretation depends upon the concentration of sugar in the blood after considerable excretion, storage and combustion of carbohydrate have taken place. Jørgensen (10) and others (11) have used intravenous glucose tolerance tests similar in principle to this one. McKean, Myers and Von der Heide (12) relied solely on changes in the blood sugar during the first hour or so after injection, and emphasized particularly the changes during the first quarter hour. Their own data reveal a marked variability in the first hour; during this period numerous changes in distribution and excretion take place which have little relationship to the utilization of glucose. It appears that at least an hour must pass before a true picture develops of the manner in which the individual handles a test dose of glucose.

There is little need to explain the advantages of

the intravenous glucose tolerance test over the oral variety. The remarkably small normal variation of the blood sugar two hours after the injection of glucose indicates the desirability of eliminating gastro-intestinal factors, since no comparable point of equal constancy can be found after oral administration (13). This point is strikingly brought out by results obtained in a patient with steatorrhea who was subjected to both an oral and to an intravenous test. The two curves are shown in Figure 2. The blood sugar remained nearly constant during the two hours after the oral ingestion of 50 grams of glucose, while it rose and fell normally following the intravenous injection of 25 grams. The discrepancy can most reasonably be attributed to defective or delayed absorption of glucose in the oral test, rather than to any incapacity to deal with the glucose after reabsorption.

A variety of disturbing factors produce large standard deviations of the mean at all points during the first hour (Table II), so that during this period it is difficult to be sure whether or not a given curve is abnormal. Certainly in no case does the "rapid fall" observed in some patients exceed

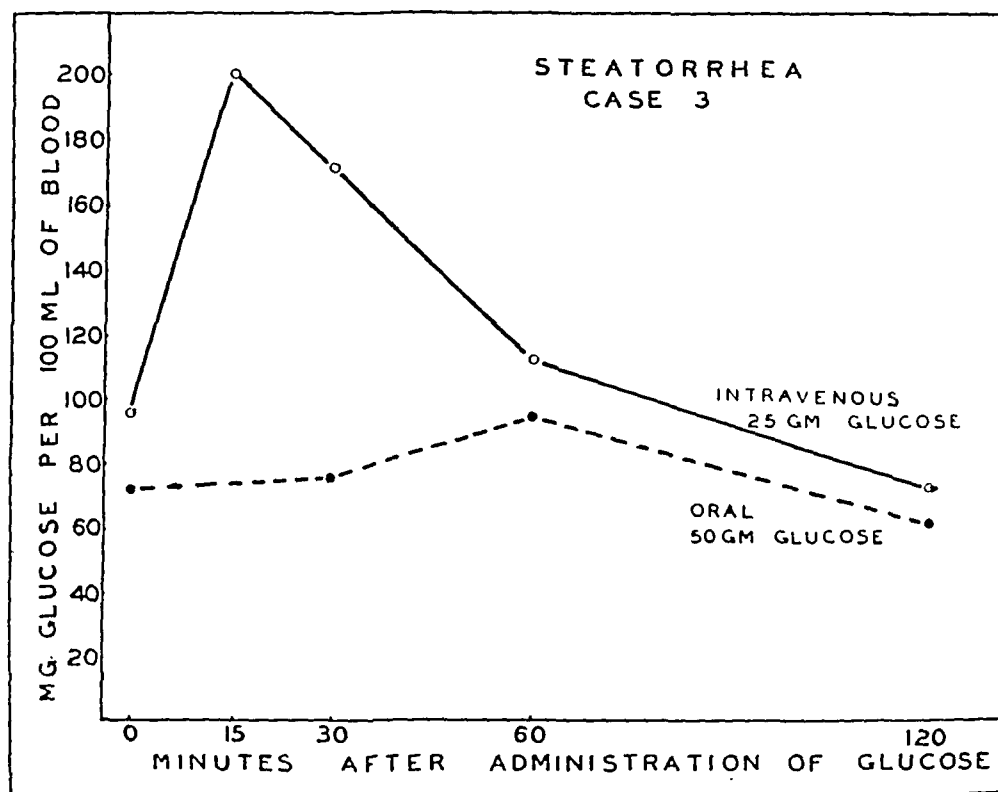


FIG. 2. COMPARISON OF ORAL AND INTRAVENOUS GLUCOSE TOLERANCE TESTS ON A PATIENT WITH IDIOPATHIC STEATORRHEA

the rate of fall of the blood sugar in some normal individuals. On the other hand, if sufficient time be allowed, the blood sugar will normally return to a very restricted range, and deviations from normal are easily demonstrated. The data presented here indicate that the value of the blood sugar two hours after injection is the most satisfactory index for differentiating between normal and abnormal curves.

Before proceeding to a discussion of Tables III and IV the term "diabetes mellitus" must be accurately defined. This term is in fact used in two senses: (1) as a disorder of metabolism; and (2) as a disease entity. In the first sense the term "diabetes" is applied to any and all subjects whose maximum attainable rate of carbohydrate combustion is less than normal. The intravenous glucose tolerance test is almost by definition diagnostic for "diabetes" in this sense, since, in general, failure of the blood sugar to fall at its usual rate means some retardation of combustion. Consequently, all patients with elevation of the blood sugar two hours after injection of glucose presumably have diabetes in this functional sense of the word. From the clinical standpoint, however, the second definition of diabetes, *i.e.*, diabetes as a disease entity, is of greater importance. Anyone with hyperglycemia, with or without glycosuria, who on the basis of past clinical experience may sometime develop certain characteristic clinical manifestations, suffers from the disease "diabetes mellitus". These manifestations include polyuria and polydipsia associated with malnutrition and, under some circumstances, ketosis and coma. These disturbances disappear under appropriate therapy. Many individuals potentially liable to the development of these difficulties may not actually suffer from them for long periods of time, if ever. A clinical diagnosis of diabetes not depending on a glucose tolerance test can be made in such persons in only two ways. First, there may be a history of clinical manifestations of diabetes in the past. Second, the individual, though symptom-free, may have hyperglycemia of so pronounced a degree that, on the basis of past experience, he is potentially liable to develop diabetic symptoms. The diagnosis of diabetes in this series was based on one of these two kinds of evidence independently of the glucose tolerance test itself. In order

to avoid a circular argument in correlating the results of the intravenous test with the diagnosis, the test itself was never used to derive the diagnoses in the left-hand columns of Tables III and IV.

From Tables III and IV it is clear that twenty-six out of thirty-one, or 84 per cent, of the patients with markedly elevated blood sugar levels after two hours had some independent evidence of the disease "diabetes mellitus". Of the remaining five patients, two had anorexia nervosa associated with extreme malnutrition, leaving only three with no evidence of disturbed carbohydrate metabolism other than the glycosuria. The analysis of Table IV indicates that the evidence for diabetes mellitus was somewhat uncertain in only four of twenty-six classified in Table III as having diabetes mellitus; in the remainder the evidence was unequivocal. The disturbed carbohydrate metabolism in the two individuals with anorexia nervosa and marked malnutrition was presumably associated with starvation (14). Obviously, these patients had "diabetes" in the functional sense of the term, but did not have the *disease* "diabetes mellitus". The importance of the definitional distinction made above is here apparent.

Of the 119 patients in whom the tests were done because of glycosuria, seventy-three had normal sugar tolerance curves (Table IV). Of these seventy-three patients, sixty-seven, or 92 per cent, had no evidence of diabetes mellitus. On the other hand, only ten of the nineteen patients with the most definite evidence of diabetes had markedly elevated sugar tolerance curves. The reason for this apparent anomaly can be found in the mode of selection of the cases, since the test was only rarely performed in clear-cut cases of diabetes. The nine patients without markedly elevated curves included the cases of so-called "acute" or "cured" diabetes. Evidence of diabetes was non-existent at the time the tests were performed, but had been unmistakable at an earlier date. A more detailed clinical account of these patients will be presented elsewhere. The tests were performed because at the time there was no available collateral evidence of diabetes. Only nine of the seventy-eight patients with no evidence of diabetes other than glycosuria had either moderately or markedly elevated curves. This group of nine



is especially interesting, since only the future can decide whether these patients will develop the disease "diabetes mellitus". In spite of these borderline cases, the conclusion is justified that a delayed fall in the blood sugar is correlated with an independent clinical diagnosis of the disease "diabetes mellitus" in about 90 per cent of the cases. The percentage would have been much higher had fewer dubious cases been included; as the figures stand, the odds are at least ten to one that a patient with a markedly elevated curve has diabetes mellitus. Conversely, the odds are ten to one that the patient does not have diabetes if the blood sugar falls to normal within two hours. In the present state of knowledge, moderately elevated curves are of little diagnostic value.

No markedly elevated curves were encountered in patients with conditions other than diabetes or suspected diabetes (Table III). Moderately elevated curves are found not infrequently in such diverse states as acromegaly, basophilism, hyperthyroidism, syncope, pancreatic disease, brain tumor, anorexia nervosa, liver disease and arthritis. In all these conditions occasional abnormalities of carbohydrate metabolism have previously been reported, usually on the basis of oral tests (1, 2). In acromegaly this impairment may be so severe as to produce the disease "diabetes mellitus" (15); the one case of this type in Table III is listed among the diabetics. In liver disease there may be a limitation of capacity to metabolize glycogen, which in turn may affect the glucose tolerance test (16). The occasional presence of a moderately elevated curve is, however, of little diagnostic value in these conditions, since at least an equal number of persons within each disease group have normal curves.

The value of this type of intravenous glucose tolerance test in the detection of pathological hypoglycemia is not great. This is in accord with the experience of Wilder (17) and others. In almost all the normal subjects the blood sugar fell below its fasting level within two hours after the injection of glucose, and in this sense some measure of hypoglycemia is physiological. The blood sugar, however, never fell low enough to be associated with symptoms. In the present series the test was carried out on fifty-six subjects suspected of hypoglycemia because of their symptoms. Four of

these had hypoglycemia at the end of two hours, including one case with true islet cell adenoma. On the other hand, the two remaining patients with islet cell adenomata (one unverified) had entirely normal curves. Furthermore, definite depression of the blood sugar at two hours was found in six patients not suspected of hypoglycemia, including two subjects with glycosuria and suspected diabetes, two with steatorrhea, one with brain tumor and one with liver disease. In these subjects no symptoms were associated with the hypoglycemia induced by the test.

Obviously, a test which is normal in two out of three cases of islet cell tumor of the pancreas is of little value in this diagnosis. This is consistent with the results obtained in this condition with the three-hour oral glucose tolerance test, for normal, delayed and accelerated curves have been reported, depending on the previous nutritional state (14). All three cases of islet cell tumor, however, developed marked hypoglycemia with severe symptoms when the period following the ingestion of glucose was prolonged for several hours. It therefore seems logical to apply a modified test if evidence of this diagnosis is sought. The modification would omit samples prior to the two-hour point; hourly samples would then be obtained for at least five hours following the injection, or until the appearance of symptoms. Wilder (17) recommends even longer periods of fasting.

The value of the present test in the diagnosis of hypoglycemia not due to islet cell tumor, but associated with symptoms, is also doubtful. In the first place, a positive result is obtained in a variety of disorders in which there are no symptoms of hypoglycemia. Secondly, a negative result has little meaning, since normal curves may be found in cases having true islet cell adenomata. The rarity in this series of hypoglycemia exceeding the normal range suggests either that the condition is rare, or that this is not the proper way to demonstrate it. The prolonged test suggested above might logically be substituted for the two-hour test whenever hypoglycemia from any cause is suspected.

The intravenous glucose tolerance test, of course, requires an operator with some skill in intravenous technique. Occasionally, local irritation along the vein follows the injection of con-

centrated glucose. These technical difficulties are, however, slight, and the advantage derived from an exact knowledge of the amount of glucose to be metabolized is considerable. Perhaps much of the controversy concerning the tolerance for carbohydrate in specific diseases would be resolved if an intravenous test such as is here described were more universally adopted.

#### SUMMARY AND CONCLUSIONS

1. An intravenous glucose tolerance test is described and normal standards are defined.

2. A single blood sugar drawn two hours after the intravenous injection of 25 grams of glucose is a reliable guide in distinguishing between benign glycosuria and diabetes mellitus. If the blood sugar is greater than 120 mgm. per 100 ml., the patient probably has diabetes mellitus, while if it is less than 100 mgm. per 100 ml., he probably does not. If it falls between 100 and 120 mgm. per 100 ml., the test is indeterminate.

3. In this series the only condition among patients without glycosuria in which the blood sugar after two hours exceeded 120 mgm. per 100 ml. was anorexia nervosa with marked malnutrition. The blood sugar fell between 100 and 120 mgm. per 100 ml. in a variety of conditions, including acromegaly, basophilism, hyperthyroidism and arthritis. Little diagnostic value could be assigned to the test in these states, since blood sugar values less than 100 mgm. per cent were equally common in each group.

4. Hypoglycemia of moderate degree, unassociated with symptoms, appeared in the majority of normal subjects. The blood sugar was normal in subjects with islet cell adenomata and subnormal in subjects without symptomatic or other evidence of endocrine disease. The two-hour blood sugar is therefore of little value in the diagnosis of pathological hypoglycemia.

The technical assistance of Nancy Marean de Fritsch, A.B., is gratefully acknowledged.

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# THE PERIPHERAL VASCULAR RESPONSE TO EXERCISE IN THE HYPERTHYROID STATE

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In the hyperthyroid state, the various compensatory mechanisms ordinarily initiated by exercise may be modified by certain factors. Of interest in this respect is the finding that the resting cardiac output (1) and rate of peripheral blood flow to at least the forearm (2) and leg (3) are markedly augmented, thus suggesting that an increased amount of blood is necessary merely in maintaining the vegetative processes. Furthermore, since hyperthyroid patients require nearly twice as many calories as normal subjects to do a definite amount of exercise, the probability exists that they are inefficient in the performance of work (4).

In the present investigation, it was considered of interest to study the local circulatory response to exercise in hyperthyroidism, in order that some information might be gained as to the adequacy of the adaptive mechanisms in this state. For this purpose the venous occlusion plethysmographic method was utilized, since it afforded an opportunity of determining the changes in a small group of muscles.

## METHOD

The study was conducted upon the forearm of 5 patients before and after subtotal thyroidectomy, the technique used in making the blood flow determinations being identical in all respects to that previously described (5). After 10 to 20 control readings had been obtained, the subject was required to perform a specified amount of work. This was accomplished by raising the pressure in a 5-gallon air-filled bottle to a definite level by means of ipsilateral manual compression of an ordinary sphygmomanometer bulb. The number of times the bulb was compressed, the period of exercise, and the height to which the pressure in the bottle was elevated were kept as constant as possible in all experiments performed on any one subject. Since the muscles utilized in this act were for the most part limited to the forearm encased in the plethysmograph, the changes in the blood flow readings could therefore be considered to represent the local circulatory reaction to the work.

Immediately upon completion of the task, forearm

blood flow determinations were obtained at 10-second intervals during the first 3 minutes, and thereafter at one-half minute periods until the blood flow returned to the previous resting level. In each instance, a graph was constructed from these figures, and by means of a planimeter the number of cc. of excess blood flow, over and beyond the previously determined average resting level, was calculated (Figure 1).

## RESULTS

With one exception (L. M.), the number of cc. of excess local blood flow elicited by a period of work was definitely greater during the hyperthyroid state than after partial thyroidectomy (Table I). A correlation was readily apparent between the magnitude of the repayment and the level of basal metabolism (Figure 1). Similarly, the duration of the increased blood flow and the height of the single maximal response were significantly less following thyroidectomy (Table I). In the case of L. M., the reading which was not in agreement with the rest of the series was obtained 53 days postoperatively, at a time when signs suggestive of early hypothyroidism were present. In respect to the group as a whole, no definite relationship existed between the magnitude or duration of the blood flow repayment following exercise and the level of the resting blood flow at the time of the test (Table I, subjects J. L. and E. M.).

## DISCUSSION

Since it was impossible to obtain accurate blood flow readings during the actual performance of exercise, the rate of blood flow in the period immediately following was studied instead. The rationale for this procedure is based on the assumption that, if the augmented circulation present during work is insufficient to meet entirely the increased demands of the tissues, a blood flow debt must be incurred; this, in turn, being repaid in the subsequent period of rest. The magnitude of the blood flow repayment would thus serve as

<sup>1</sup> Aided by the Samuel and Regina Kuhn Fund.

an index of the circulatory response during the exercise. On such a basis, the foregoing results lend themselves to certain interpretations.

The finding of a much larger blood flow repayment in the hyperthyroid state than in the period following operation implies either that the compensatory mechanisms present during exercise are inadequate, that the blood flow requirement per unit of work is much greater than normal, or that both of these factors are operating. The fact that the cardiovascular system in the resting hyperthyroid patient is already functioning at a greater level of effort, analogous to that of a compensated heart with diminished reserve, suggests that the increased post-exercise response is due

at least in part to inadequate adaptive mechanisms. Further, the finding that a definite relationship exists between the rate of metabolism and the magnitude of the blood flow repayment implies that the muscles of hyperthyroid patients require a greater than normal quantity of oxygen and food-stuffs in the performance of a specified amount of work. In other words, the local blood flow demands in exercise appear to be related to the metabolic level of the organism as a whole.

It would seem, therefore, that exercise places a severe load upon the circulation in hyperthyroidism, since a greater than normal blood flow requirement must be satisfied by a cardiovascular system already geared to a higher level of effort

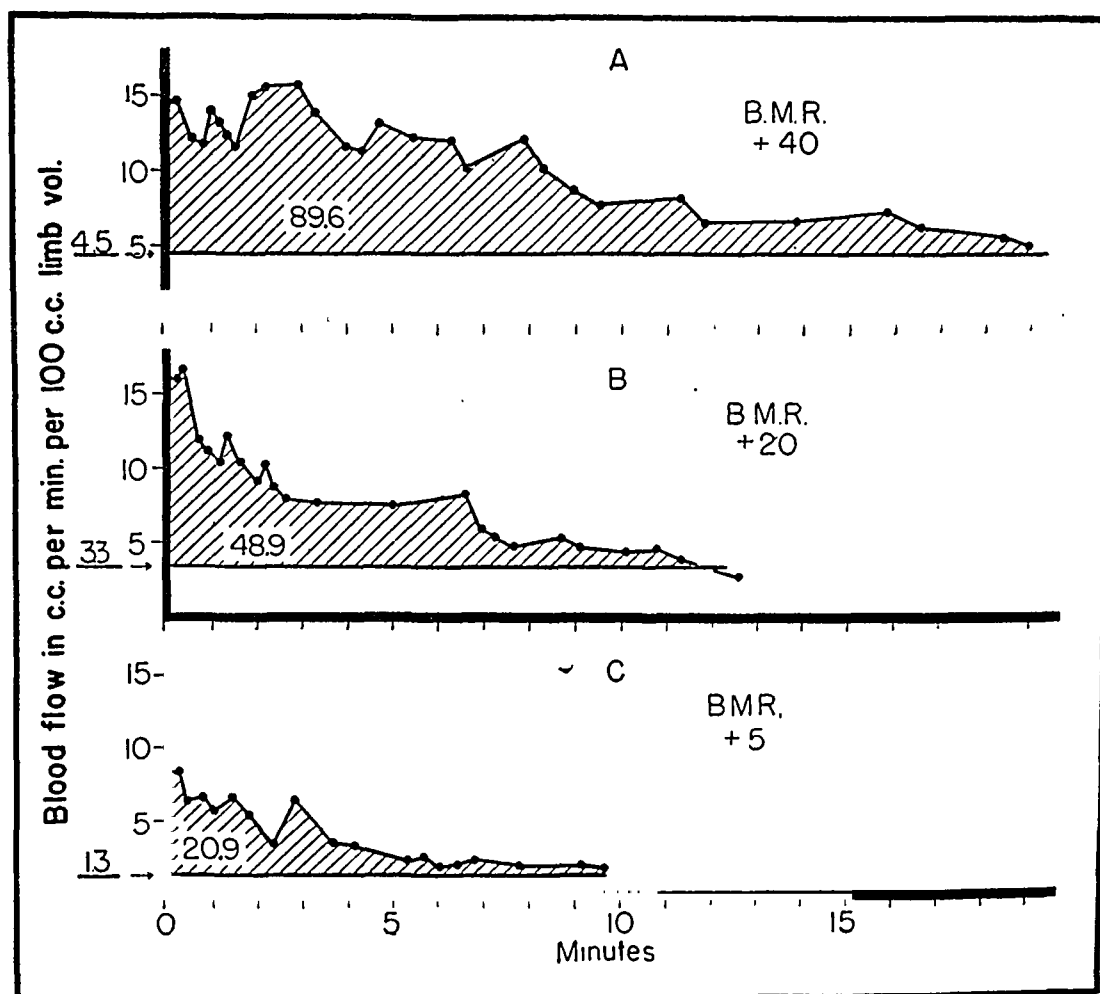


FIG. 1. BLOOD FLOW RESPONSE IN FOREARM TO A SPECIFIED AMOUNT OF EXERCISE  
SUBJECT E. M. (TABLE I)

A. 5 days before operation. Estimated basal level in period immediately following exercise, 4.5 cc. per minute per 100 cc. limb volume (equivalent to resting control blood flow before exercise). Excess blood flow elicited by the work, 89.6 cc. per 100 cc. limb volume. B. 4 days postoperatively. Estimated basal level, 3.3 cc. Excess blood flow, 48.9 cc. C. 28 days postoperatively. Estimated basal level, 1.3 cc. Excess blood flow, 20.9 cc.

TABLE I  
Blood flow response to exercise in the forearm

Subject	Relationship to operation	Exercise*	Control flow	Repayment†	Maximum single response to exercise	Time of maximum response	Total duration of response	Basal metabolic rate
	<i>days</i>	<i>mm. Hg</i>				<i>seconds</i>	<i>minutes</i>	
J. L.	2 preoperative 10 postoperative	70 70	4.2 4.1	55.7 26.0	13.5 8.6	45 30	16 14.5	+24 +12
M. B.	12 preoperative 35 postoperative	40 40	3.6 1.2	33.0 18.0	10.7 6.9	30 10	19 10	+50 0
E. M.	5 preoperative 4 postoperative 28 postoperative	45 45 45	4.5 3.3 1.3	89.6 48.9 20.9	15.1 16.3 8.2	180 20 10	19 13 10	+40 +20 + 5
J. P.	1 preoperative 4 postoperative	60 60	5.3 2.5	42.7 22.6	11.8 8.3	40 10	20 10	+44 +15
L. M.	7 preoperative 8 postoperative 53 postoperative	50 50 50	5.8 4.2 1.2	42.7 35.4 41.9	13.1 10.5 8.2	100 60 25	15 15 10	+44 +23 -13

\* Height of pressure in 5-gallon bottle.

† Total number cc. excess blood flow per 100 cc. limb volume elicited by exercise.

Blood flow figures expressed in cc. per minute per 100 cc. limb volume.

during rest. In view of this, the fact that the hyperthyroid state is commonly associated with the symptoms of muscular weakness and fatigability is readily understandable.

#### SUMMARY AND CONCLUSIONS

1. The post-exercise blood flow repayment was generally found to be much greater in the hyperthyroid state than in the period following subtotal thyroidectomy.

2. A correlation was apparent between the level of oxygen consumption and the magnitude of the excess blood flow elicited by the exercise.

3. Exercise places a much greater load upon the circulation in hyperthyroidism than in the normal state.

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# STUDIES ON PAIN. OBSERVATIONS ON PAIN DUE TO LOCAL COOLING AND ON FACTORS INVOLVED IN THE "COLD PRESSOR" EFFECT

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When a subject's hand is immersed in cold water two phenomena are known to occur. The subject experiences pain and an elevation of blood pressure. The latter phenomenon has been used as the basis for the Hines-Brown "cold pressor" test (1). The occurrence of pain has received little attention, and is alluded to only incidentally in the articles on the "cold pressor" test. Pain, however, has been suspected of being concerned in the production of the hypertension (2). In the present work on pain due to local cooling, we have found direct correlation between the degree of cooling, the intensity of pain induced by the cold, and the height to which the blood pressure rises.

Lewis (3), in an analysis of the vascular reaction to cold, noted that in fingers immersed in cold water from 0° C. to 18° C. a painful aching occurred "soon" after immersion. No attempt was made to study the nature of the pain. The burning pain produced on the skin of the arm by a dry ice stimulator has been studied (4). The pain was shown to be adaptable and, contrary to Goldscheider's theory that pain is an exaggerated pressure sensation, it was shown to be a separate sensory function. The mechanism of production of the pain, however, was not investigated.

The aim of this communication is to report on a study of the phenomenon of pain due to local cooling, which, for convenience, we call "cold pain". The pain is conveniently produced and, owing to its predictable character, lends itself to analysis.

## METHOD

The subject was placed in a reclining position on a cot beside an earthenware crock holding 20 liters of water which was stirred vigorously by a propeller attached to a small motor. Because of the large volume, the bath could be maintained at constant temperature for the duration of an experimental observation without the

aid of a thermostatic device. The left hand of the subject was plunged into the water up to the wrist. The time of onset of pain was recorded and an attempt was made to estimate the intensity of pain being suffered at a given moment.

While these observations were being made, the blood pressure in the opposite arm was estimated by means of a mercury sphygmomanometer at as frequent intervals as possible. The skin temperature of the immersed hand was determined continuously by the use of a copper and constantan thermocouple fixed to the pad of the terminal phalanx of the middle finger. The amplitude of pulsations of the digital artery was also recorded by using a small glass plethysmograph fitted to the index finger of the immersed hand. The plethysmograph was connected by an air system to a tambour on which was mounted a small mirror. Movements of the latter were recorded on a camera by reflecting a beam of light on the moving film. Extraneous factors which are known to influence the amplitude of pulsation of the digital artery (5 to 13) were all carefully controlled.

The results of a series of 54 experiments which provide 121 observations are reported. The observations were made mainly by the authors upon one another, since uninterested subjects could scarcely be called upon to undergo the necessary amount of physical discomfort.

## OBSERVATIONS

### *I. Sensations induced by local cooling*

*A. Deep aching pain and its "adaptation".* Immersing the hand in water warmer than 18° C. caused no pain, but at 18° C., and slightly below, there was a fleeting deep ache which occurred after the hand had been immersed about 60 seconds, and then promptly ceased. At progressively lower temperatures the pain had its onset sooner and was more intense, always reaching its maximum at about 1 minute. It then began to subside, and in 4 to 5 minutes was no longer perceived. The character of the pain was aching; there was a feeling as if the hand had been crushed. The distribution was generalized and deep throughout the immersed hand, and the pain was perhaps most intense on the radial side. It was continu-



ous and non-pulsatile. Exercise of the fingers or movement of the hand did not influence the character or intensity of the pain. There was no tenderness in the hand since it could be struck against the side of the bath without altering the sensation of pain. Sixty seconds after immersion the peak of pain intensity was reached. This was followed by amelioration, and finally cessation of the pain. The disappearance of pain while the hand is still immersed in the water we have called "adaptation".

A barely perceptible painful sensation experienced as a deep ache in the immersed hand was designated as threshold pain. Additional increments in pain intensity were indicated in terms of pulses, 8+ being used for the pain of greatest intensity experienced. The distribution of pain as high as 2+ was confined to the hand. At

3+, however, there occurred a radiation of the ache up the inner aspect of the arm. At 4+ pain was felt in the axilla. At this intensity the subjects usually showed a reaction to the stimulus characterized by rapid irregular respirations, as well as by adventitious movements of the feet. At 5+ the restlessness was replaced by writhing movements and the subject's face betrayed suffering. From 6+ to 8+ the pain provoked perspiration and approached the unbearable level. During the first minute after immersing the hand in cold water it was quite clear that the pain was becoming more and more severe. Each time a definite increase in pain intensity was felt the subject called out an additional plus. Similarly, as adaptation occurred after one minute and the pain was clearly becoming less intense, the subject called out one less plus at each perceptible de-

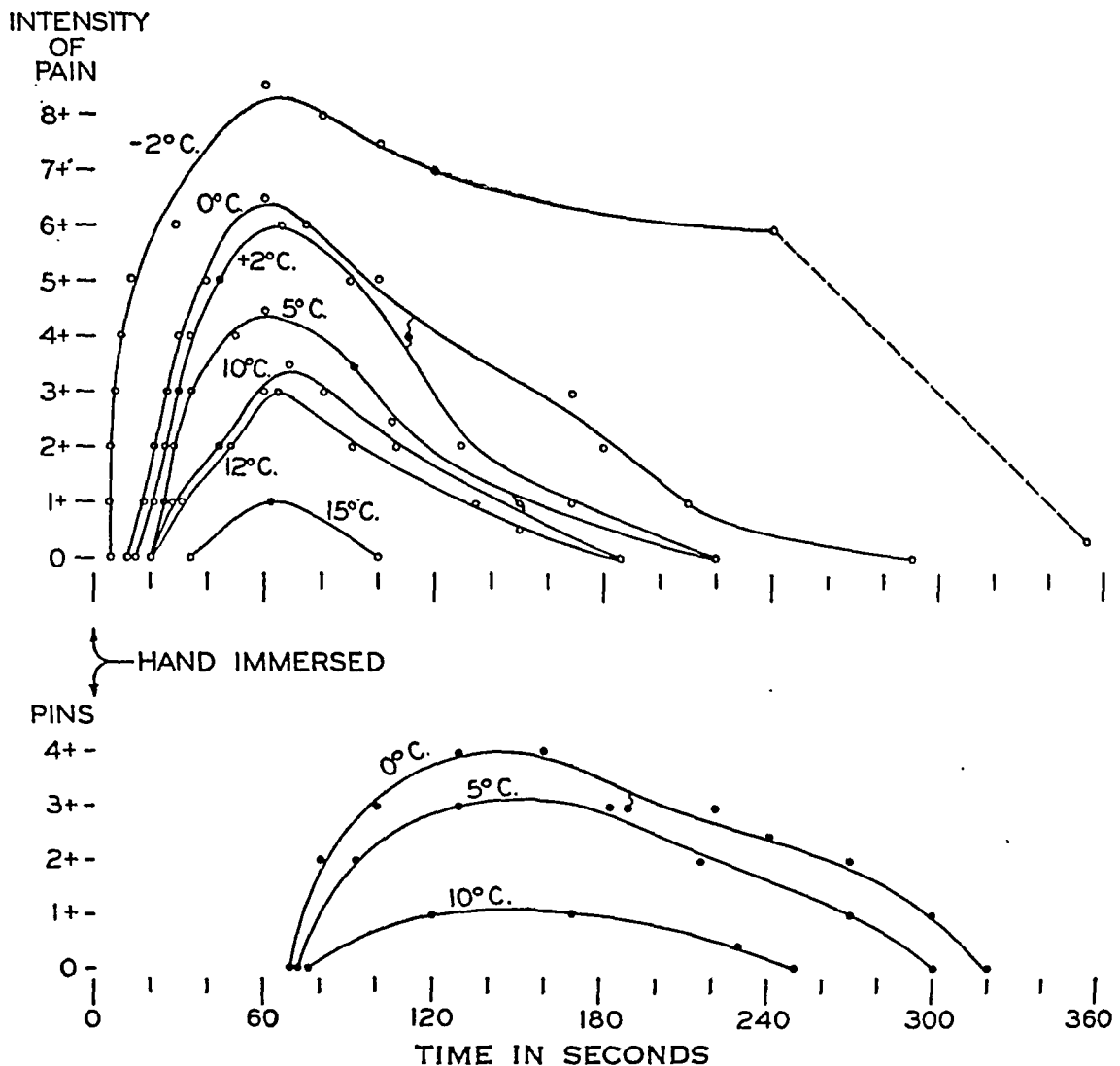


FIG. 1. THE COURSE OF THE ESTIMATED INTENSITY OF PAIN AND "PINS AND NEEDLES" SENSATION EXPERIENCED WITH VARIOUS STRENGTHS OF COLD STIMULUS

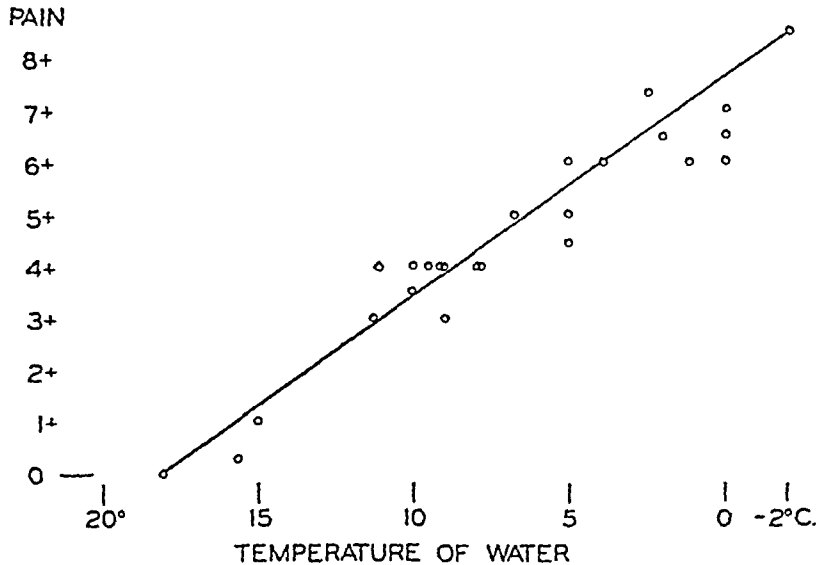


FIG. 2a. THE ESTIMATED PEAK OF PAIN INTENSITY PLOTTED AGAINST THE STRENGTH OF THE COLD STIMULUS

crease in pain intensity. The values thus established were roughly reproducible at any time. A subject immersing his hand in water of unknown temperature consistently called out the same number of pluses as had been previously assigned to that particular temperature. Therefore, although not precise, this method has proved useful here as in other studies of sensation (14, 15). Figure 1 shows the course of estimated pain intensity in the hand immersed in water at different temperatures. Figure 2a shows maximal pain intensity plotted against water temperature. It is apparent that pain intensity as estimated increases uniformly with the strength of stimulus measured in terms of temperature of the water bath. It is also

of interest that the line points directly at 18° C. which, as noted above, was found to be the highest temperature at which this variety of pain occurs. In Figure 2b the total area under the pain curves at various temperatures is plotted against the temperature of the bath. The total amount of pain experienced also increases uniformly with the strength of stimulus.

*B. "Pins and Needles" sensation.* The sensation of "pins and needles" occurred only in water colder than 12° C. The sensation was felt in the entire immersed hand from 60 to 90 seconds after immersion. This sensation, occurring shortly after the peak of the aching pain, steadily increased in intensity as the pain decreased. The

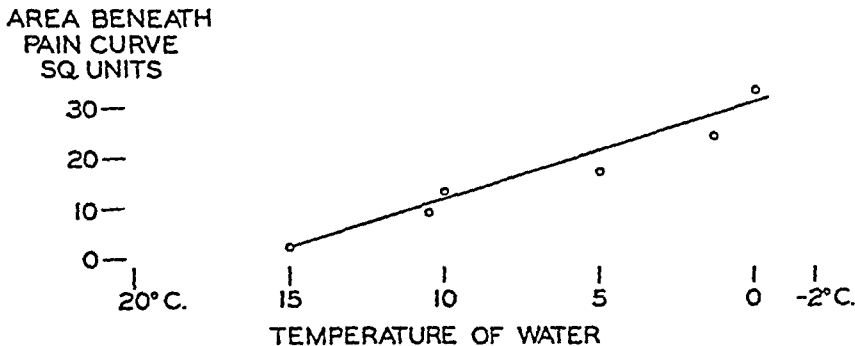


FIG. 2b. THE TOTAL AREA BENEATH THE CURVES OF PAIN INTENSITY IN FIGURE 1 PLOTTED AGAINST STRENGTH OF COLD STIMULUS

lower the bath temperature, the more intense was the prickling. Like pain, this sensation ran a cycle of aggravation and then adaptation, until at the end of 8 to 10 minutes the immersed hand felt only the cold of the water (Figure 1). The sensation of touch and of pain to pin prick, however, as well as motor power in the fingers, remained intact. This "pins and needles" sensation, when intense, caused a great deal of discomfort to the subject. At  $-2^{\circ}\text{C}$ ., although the aching pain had been tolerated well past its peak of severity, the subject was forced to withdraw his hand owing to the suffering imposed by the growing intensity of the "pins and needles" sensation.

*C. "Adaptation".* If the hand were withdrawn before the pain had reached its maximum intensity, there was a momentary twinge of extra pain immediately after withdrawal. If the hand were withdrawn at any time before complete "adaptation" had taken place and then at once re-immersed, the pain would recur with nearly as great an intensity as occurred with the original immersion. If, however, the hand were left in until "adaptation" had taken place, it could be withdrawn and re-immersed in the cold water without pain being experienced (Figure 3). This adaptive effect which protected the hand from pain diminished as the skin temperature was allowed to

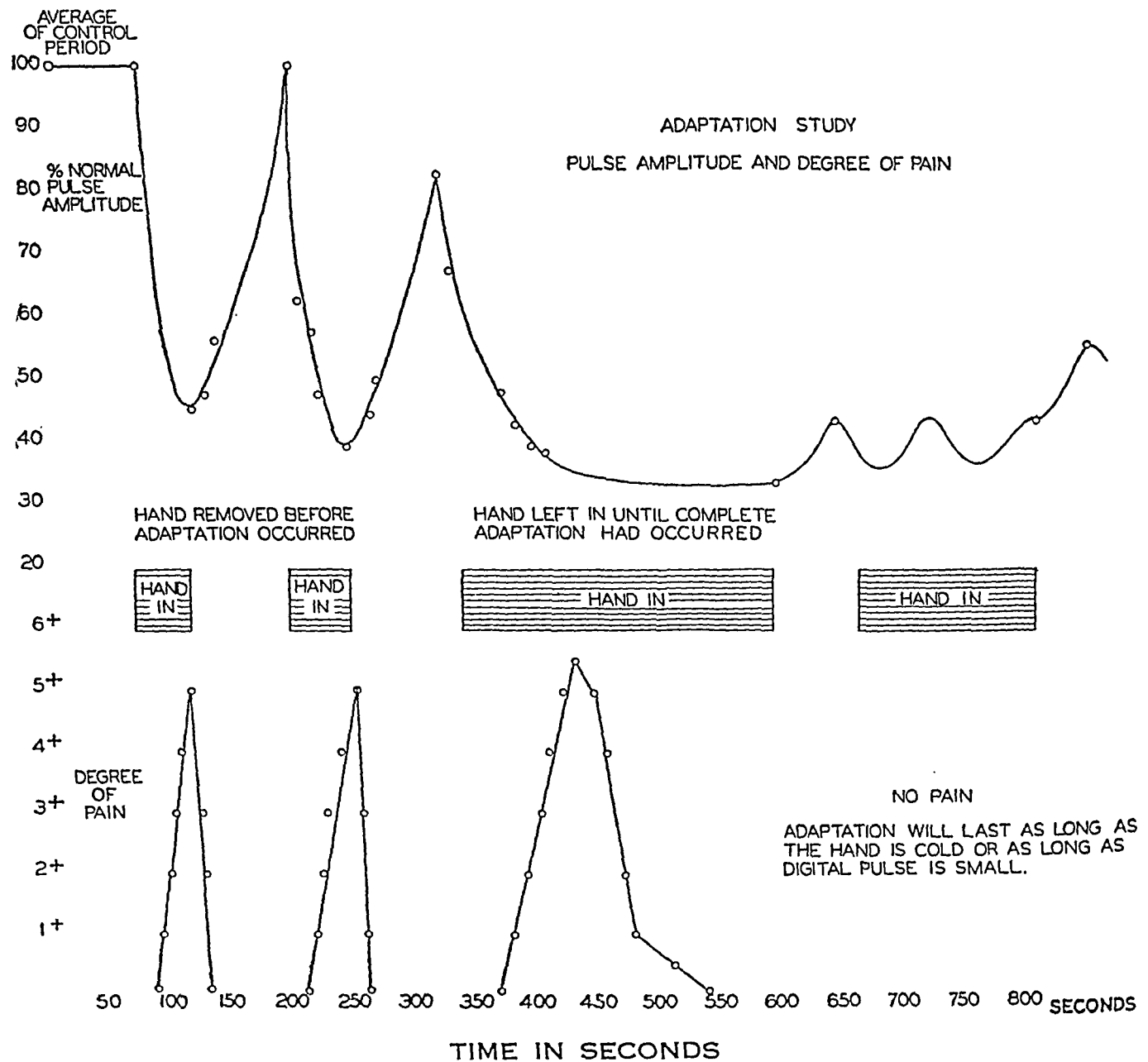


FIG. 3. STUDY OF ADAPTATION. THE RELATIONSHIP BETWEEN THE TEMPORAL COURSE OF PAIN SENSATION, ITS "ADAPTATIONS" AND THE AMPLITUDE OF PULSATION OF THE DIGITAL ARTERY

rise. There was partial protection, however, until the temperature of the hand reached 33° C. At this point, an immersed hand would feel the usual intensity of pain regardless of how quickly or slowly it had been warmed. "Adaptation" was completely effective only for the temperature of the water in which the hand was adapted, or for higher temperatures. Thus, if a hand adapted in water at 10° C. were withdrawn and plunged directly into water at 5° C. the cycle of pain would occur as usual, the onset being somewhat delayed and the intensity of pain somewhat less than it would have been had a warm hand been immersed.

## II. Spatial summation

When applied to a sensation, the term "spatial summation" means that the larger the area stimulated, the weaker would be the stimulus necessary to produce the sensation in question. Hardy and Oppel (16) demonstrated spatial summation for heat and cold sensations on the forehead. That is, as the area exposed to the heat radiation was increased, the strength of stimulus necessary to evoke sensation decreased almost in proportion. Hardy, Wolff and Goodell (17) found that pain induced by radiant heat on the forehead did not have this property of spatial summation. With the water-bath at 18° C., as already pointed out, barely detectable pain occurs in an immersed hand. When one finger was immersed, pain of the same intensity occurred. Immersing one finger at 0° C. brought on pain of intensity equal to that experi-

enced when the whole hand was immersed. This type of pain, then, like burning pain, fails to show the effect of spatial summation.

## III. "Cold Pain" in parts other than the hand

The hand is not the only part of the body in which pain can be induced by local exposure to cold. The feet and legs were similarly sensitive to cold, as was the face, tongue, scrotum, etc. When the vertex of the head was dipped in cold water, pain was induced in the vertex following the pain cycle described for the hand. The sensation spread down the back of the head and through the temples, and appeared to be more intense than that experienced for the hand under like conditions. The highest temperature at which the headache could be induced, however, was 18° C.

In two sites on the body it was found that pain could not be induced in our subjects by cold water. These were the lobe of the ear and the glans penis. The explanation for these exceptions is not clear at present.

## IV. The behavior of skin temperature

The skin temperature fell off sharply upon immersion. The rate of cooling of the skin was rapid for the first minute. By this time 90 to 95 per cent of the total fall in skin temperature had occurred and cooling continued at an increasingly slower rate (Figure 4).

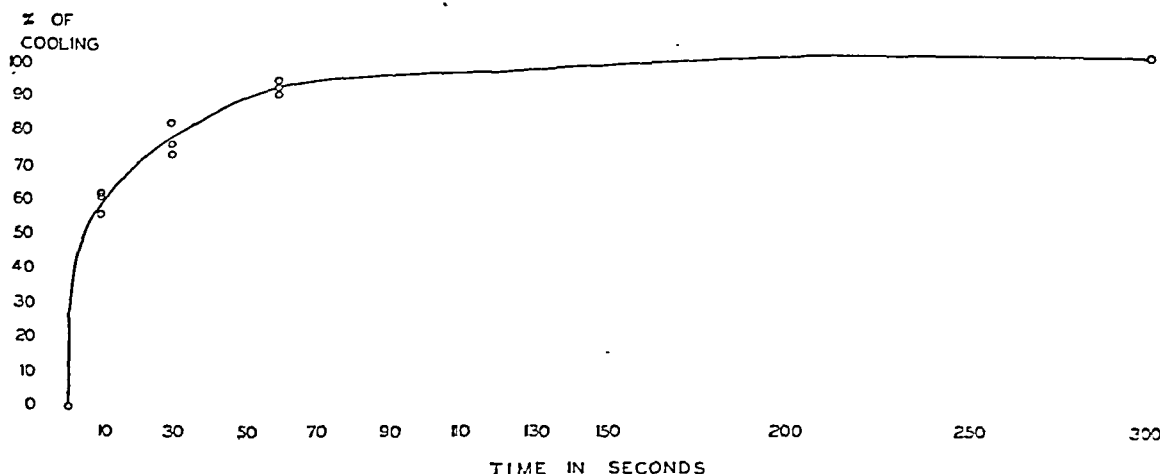


FIG. 4. THE COURSE OF SKIN TEMPERATURE SHOWING THE RATE OF COOLING AT VARIOUS BATH TEMPERATURES

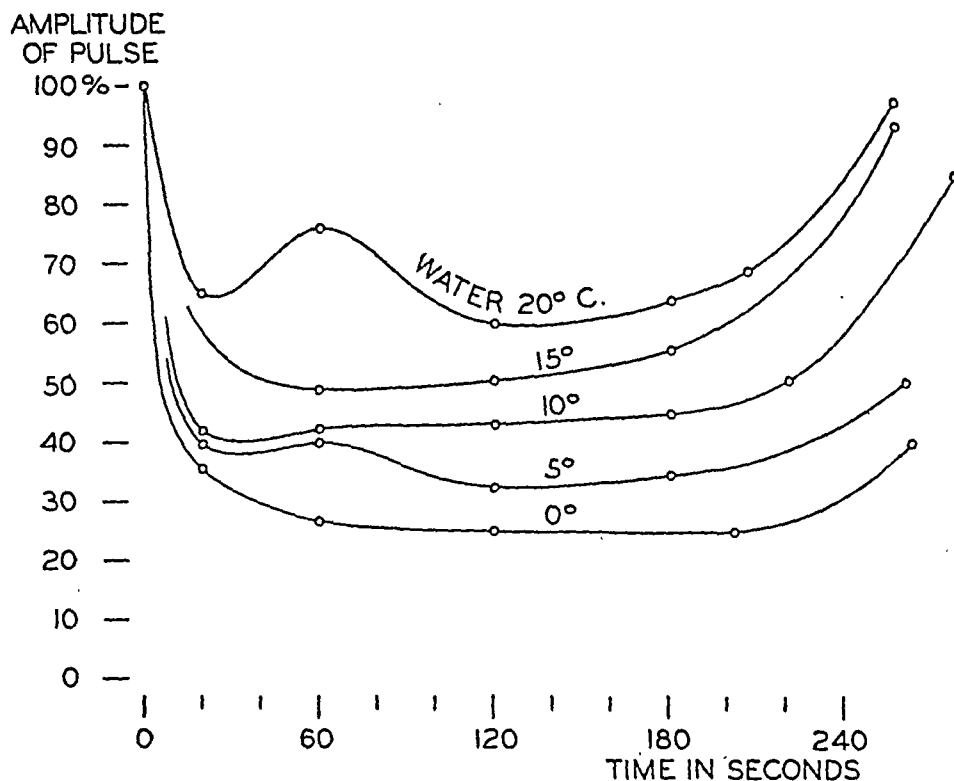


FIG. 5. THE COURSE OF SKIN TEMPERATURE AND AMPLITUDE OF PULSATION OF THE DIGITAL ARTERY WITH THE LEFT HAND IMMERSSED IN WATER AT VARIOUS TEMPERATURES

#### V. Behavior of the digital pulse

The amplitude of the digital pulse decreased suddenly upon immersion of the hand (Figure 5), reaching its minimum at the time when pain in the hand was at a maximum. Following this there was a gradual return of the amplitude toward normal. This effect was most evident at temperatures of 9° C. or higher. Figure 6 illustrates the relationship between pain, "pins and needles" sensation and the amplitude of pulsation of the digital artery, with the hand immersed at 9.5° C. The pulsations began to decrease in amplitude directly after immersion of the hand. The pulse waves of minimal amplitude correspond in point of time to the maximum intensity of pain in the hand. As the pain intensity began to decrease and the "pins and needles" sensation appeared, the amplitude of pulsation of the digital artery increased again, reaching its former level after the hand had completely adapted to the "pins and needles" sensation. At temperatures below 10° C. the intense cold usually appeared to hold the arteries more or less tightly constricted, thus masking the vasodilator effect apparent at higher temperatures (Figure 5).

Figure 7 illustrates a simultaneous recording of pulsations in the immersed fingers and the fingers of the opposite hand. The sharp decrease in amplitude of pulsation of the digital artery in the painful hand was closely paralleled by the tracing of the opposite hand. In the latter, however, there occurred shortly a vasodilator effect which brought the amplitude of pulsations back to normal.

#### VI. Behavior of blood pressure

Ten to 60 seconds after immersion of the hand in cold water, and approximately coincident with the onset of pain, a sharp rise in blood pressure occurred. This reached its maximum at about the point of maximum pain. With the onset of "adaptation" the level of blood pressure declined slowly and returned to normal after the pain and "pins and needles" sensation had disappeared. When the adapted hand was withdrawn and promptly re-immersed, there was no recurrence of blood pressure elevation just as there was no recurrence of pain. Like pain, the blood pressure elevation bore a direct relationship to the degree of local cooling (Figure 8). No elevation of blood pressure occurred in water warmer than 18° C.

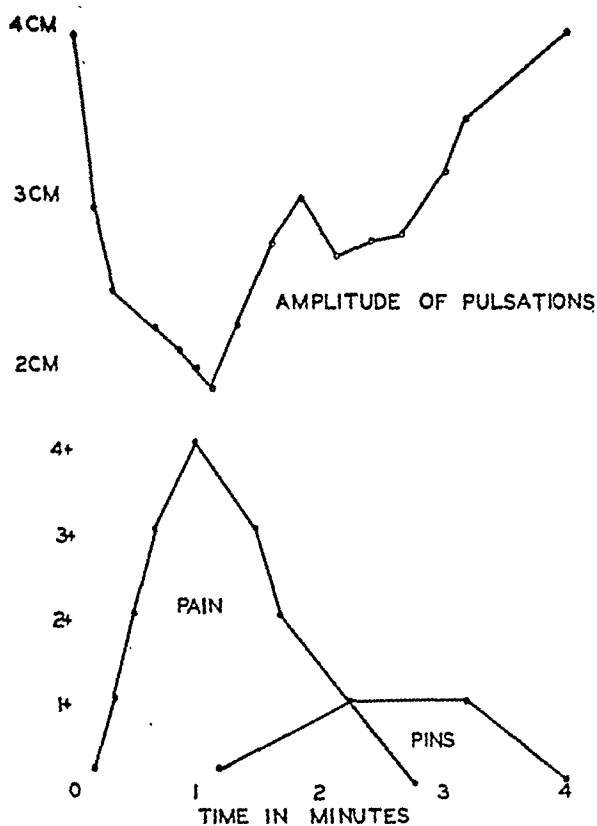


FIG. 6. RELATIONSHIP OF THE AMPLITUDE OF PULSATIONS OF THE DIGITAL ARTERY TO THE OCCURRENCE OF PAIN AND "PINS AND NEEDLES" SENSATION AT 9.3° C. The return to normal amplitude of pulsation was unusually rapid and complete in this record.

#### VII. The effect of ischemia

Using a sphygmomanometer cuff maintained at a pressure of 200 mm. of mercury, the blood supply to the arm was interrupted for 10 seconds, 5 minutes, 15 minutes, 35 minutes, and 45 minutes before the hand was immersed. The inflated cuff was left in place during the period of immersion while observations were recorded. The pain and "pins and needles" sensation occurred at their usual times, both with somewhat diminished intensity. "Adaptation" took place as usual. In the experiment in which the circulation was cut off for 45 minutes, it was noted that touch sensation, cold sensation and pain to pin prick had gone entirely from the hand before immersion (14) but, in spite of this, "cold pain" occurred as usual when the hand was immersed.

*Comment.* It is known that, when anesthesia is induced by interrupting the blood supply to a part,

the large fibers of class A are the first which cease to conduct impulses and that, as the ischemia is continued, nerve conduction stops in progressively smaller fibers until the smallest non-myelinated fibers of class C are the last to drop out (18). Cocaine anesthesia, on the other hand, has been shown to affect nerve fibers in just the opposite sequence (19), namely, the small class C fibers are the first ones blocked and the largest fibers of class A the last. Accordingly, since "cold pain" from the above experiment in which the area was rendered ischemic appeared to be conducted along the smallest fibers of the nerves, it was determined to confirm this observation by testing the effect of procaine anesthesia.

#### VIII. Effect of procaine

Three cubic centimeters of a 1 per cent solution of procaine hydrochloride were injected around the left ulnar nerve, and 30 seconds after the injection was completed the fourth and fifth fingers of the left hand were immersed in water at 10° C. Scarcely any "cold pain" appeared. Nevertheless, all other sensory modalities, including pain to pinching and pin prick, were intact at this time. Later, an incomplete and transient anesthesia developed in the ulnar distribution, but it had been possible first to eliminate "cold pain" while pin prick and deep pressure pain were still appreciated.

#### IX. Effect of sympathectomy

That this modality of "cold pain" is mediated by small non-myelinated fibers of class C appeared to be indicated from these data. Other fibers of similar size are the sympathetic vasomotor fibers. In order to determine any possible influence of these upon the phenomenon of "cold pain", a patient who had had the cervical sympathetic ganglia on the right removed within the previous four weeks for intractable asthma was used as a subject. It was found that his right hand was markedly warmer than his left. The completeness of sympathectomy was demonstrated by comparing the change in skin resistance in the two hands upon the application of a nociceptive stimulus. Repeatedly there was a marked change in resistance on the left and none at all on the right. When the left arm was immersed in cold water, the usual cycle of pain and blood pressure eleva-

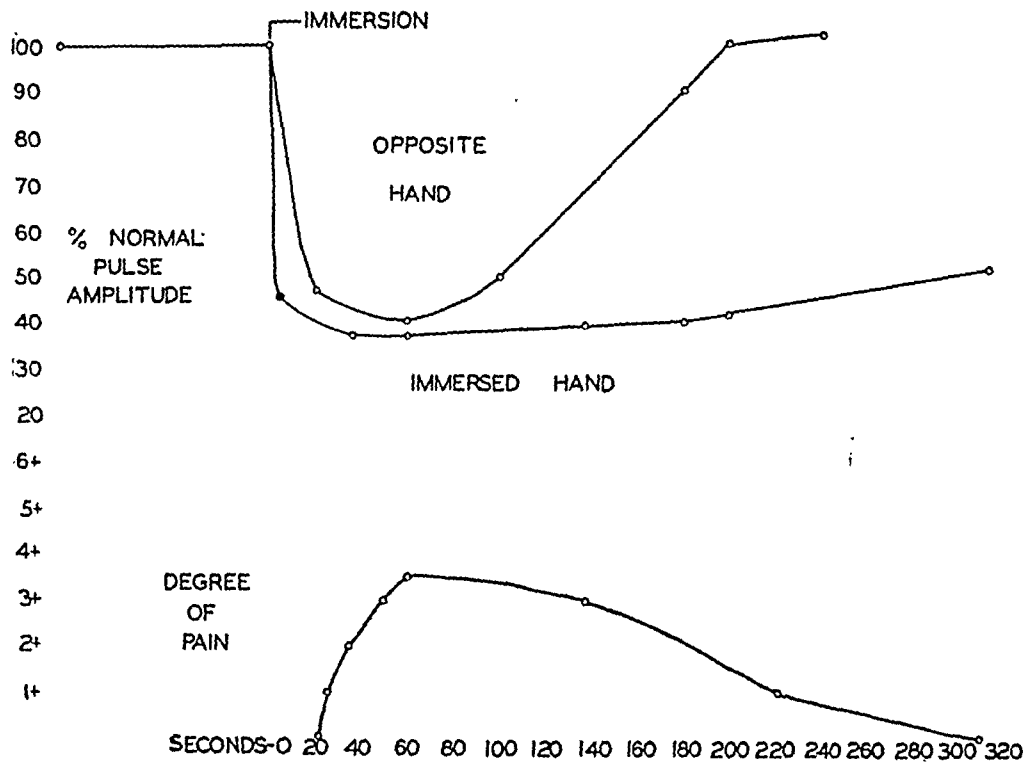


FIG. 7. A COMPARISON OF THE AMPLITUDE OF PULSATION OF THE DIGITAL ARTERY IN THE IMMERSSED HAND WITH THAT OF THE OPPOSITE HAND

DIASTOLIC

B.P. RISE

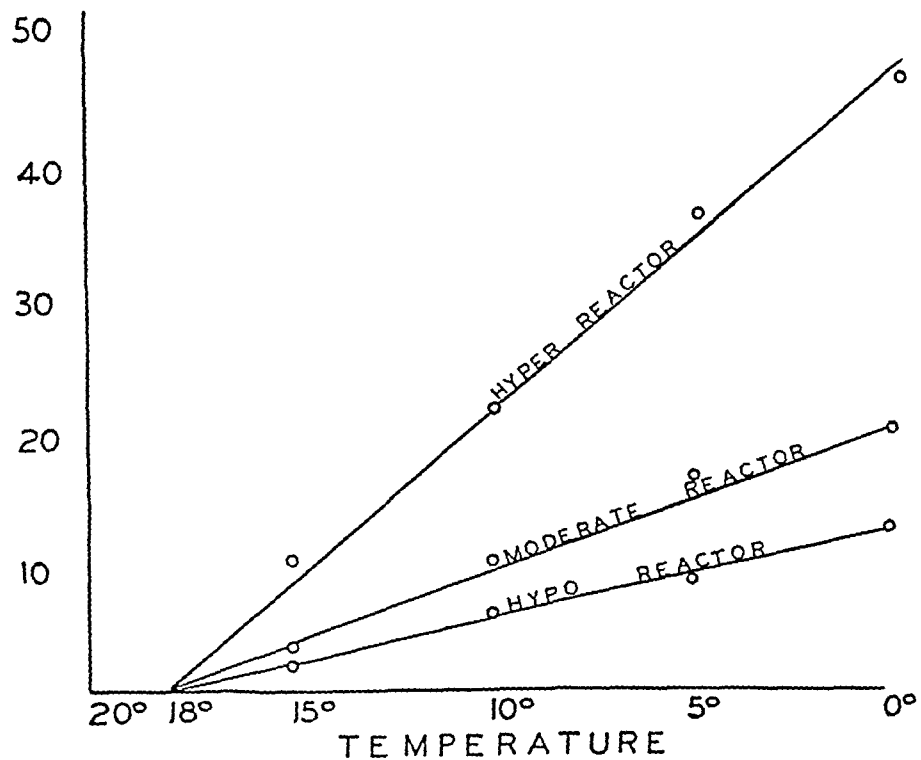


FIG. 8. RELATIONSHIP BETWEEN THE BLOOD PRESSURE ELEVATION AND THE STRENGTH OF COLD STIMULUS IN DIFFERENT INDIVIDUALS AT VARIOUS TEMPERATURES

tion occurred. When the right hand was immersed, however, the pain was much more severe and the elevation of blood pressure much more marked. At 10° C., for example, there was a diastolic rise of 10 mm. of Hg when the left arm was immersed and 20 mm. of Hg when the right arm was immersed. When the circulation to the arm was interrupted by a sphygmomanometer cuff, the effects were not altered.

### *X. The effect of slow cooling*

If the hand were immersed in water at 20° C., a temperature at which no pain could be induced, and the temperature of the water slowly lowered by successive steps to 0° C. (over a period of more than 60 minutes), no pain was felt. The sensation of "pins and needles", however, occurred at about 12° C. whether the cooling was slow or rapid, and became more intense with further cooling, adapting slowly at each new temperature. The actual intensity of "pins and needles" sensation was not as great with slow cooling as it was at comparable temperatures when the hand was cooled rapidly. The slower the cooling, the less marked was the sensation, and it is quite possible that if the hand were cooled slowly enough the sensation would not appear at all.

### *XI. The effect of successive cooling of parts*

One hand was plunged into cold water where it was allowed to remain until complete "adaptation" had taken place. Then the other hand was immersed. The usual cycle of pain occurred in the freshly immersed hand without any pain occurring in the already adapted hand. After the second hand was thoroughly adapted, one forearm was introduced. Again pain was felt in the newly immersed part but not in the hands. Similarly, after submerging the other forearm, only that part experienced pain while the other members were "adapted".

### *XII. The effect on "cold pain" of agents which alter the contractile state of arteries*

*A. The effect of vasodilator agents.* The contents of an ampule of amyl nitrite was inhaled on two occasions while the hand was immersed in cold water. There was no detectable effect on the pain. One milligram of histamine injected subcutane-

ously was similarly unavailing in modifying the pain caused by cold, although the subject became flushed as a result of peripheral dilatation.

*B. The effect of vasoconstrictor agents.* Pitressin, 1 cc., was given hypodermically to 3 subjects to determine the effect of strong vasoconstriction on the intensity and duration of "cold pain". Readings were made 15 minutes, 45 minutes, 1½ hours and 2 hours after injection. It was found that both intensity and duration of pain were increased by 50 per cent. The maximum effect was noted at 45 minutes. After 2 hours the effect had disappeared.

The fact that pitressin brought about an increase in intensity and duration of "cold pain" suggests that vasospasm may be a factor in the production of "cold pain". Ray and Wolff (20), however, in experiments on the pain sensitivity of cranial arteries, brought about a brisk vasoconstriction by painting epinephrine directly on a cranial artery without inducing pain. Yet an epinephrine pack placed in the nose is known to cause intense pain. We found that this pain could be greatly accentuated by breathing cold air and nearly completely relieved by breathing warm air. It may be that here one is dealing with a pain arising from vasoconstriction which is relieved by the vasodilator influence of warmth.

In order to test this possibility further, it was determined to induce a powerful vasoconstriction in a hand still immersed in cold water but already adapted to "cold pain". Accordingly, the left hands of the subjects were immersed in water at 10° C. and, after "adaptation" was complete and the blood pressure had returned to its control level, 0.00025 gram epinephrine was injected rapidly intravenously. Almost at once there was a sudden and dramatic rise in the systolic blood pressure to more than 200 mm. Hg. At this time the subjects were pale, weak, and dyspneic, with accelerated pulse and respirations. The mouth was dry with an unpleasant, faintly metallic taste in it, and soon a moderately severe centrally placed headache appeared. The adapted hand in the water at first tingled but there was no "cold pain"; after the blood pressure had passed its peak, the tingling was replaced by "cold pain". The latter persisted until the blood pressure had fallen nearly to normal. Then the pain gave way to "pins and needles", as usual, and finally, as the blood pres-



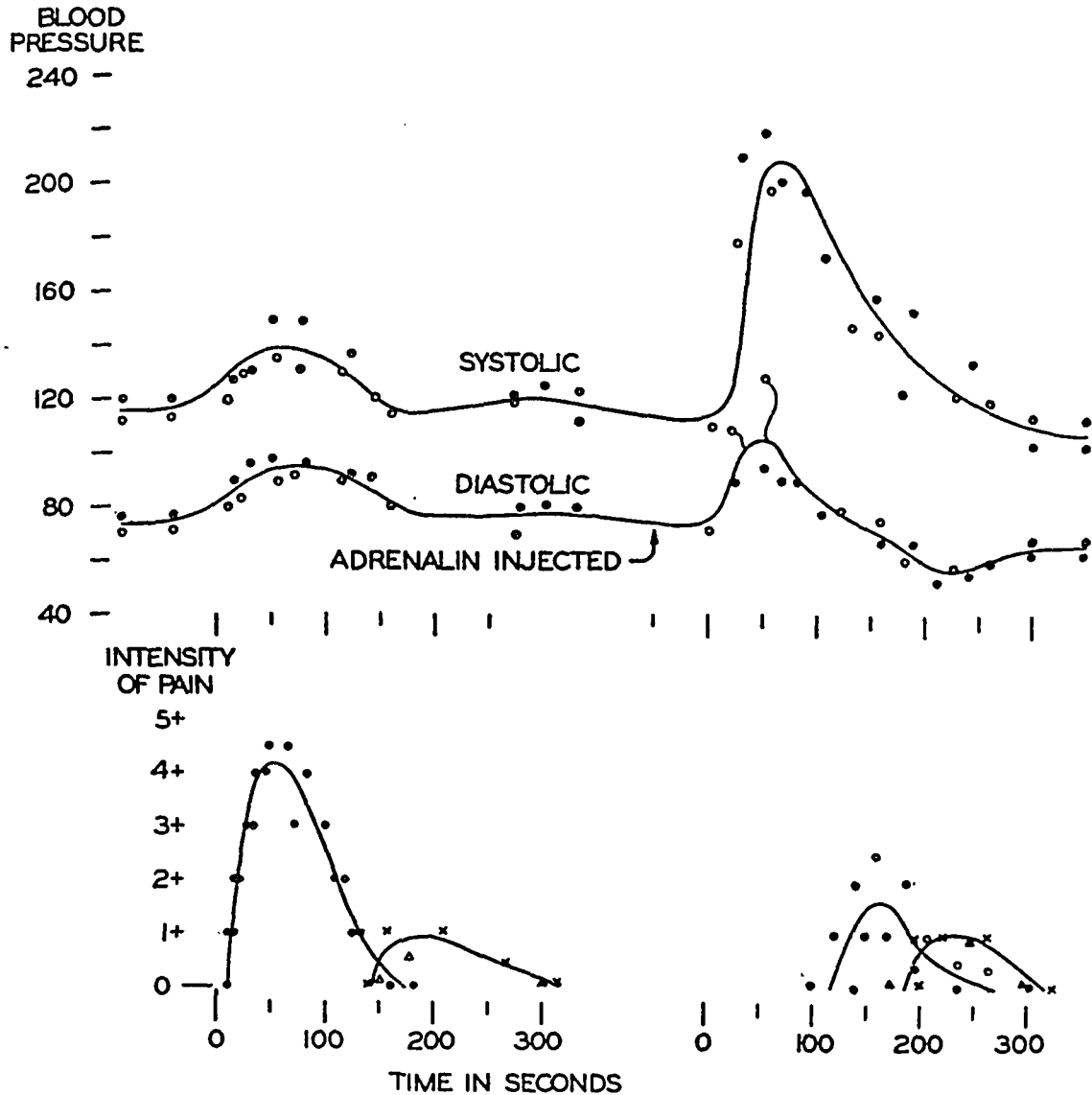


FIG. 9. PAIN INDUCED IN A HAND COMPLETELY ADAPTED IN WATER BATH AT 10° C. BY THE INJECTION OF  $\frac{1}{4}$  MG. OF EPINEPHRINE INTRAVENOUSLY

sure returned to normal, that sensation ceased (Figure 9).

*Comment.* Having been able to reproduce pain similar to "cold pain" in this way, one is still not entirely clear as to whether or not this effect was brought about by vasoconstriction or by some other effect of epinephrine. The effects of epinephrine upon deep-lying blood vessels are not entirely agreed upon by investigators, and it is quite possible that the drug effected a warming of the deeper structures of the hand and abolished "adaptation" by increasing the thermal gradient, thus again rendering the hand susceptible to "cold pain" from the water in which it was immersed.

#### DISCUSSION

From the above data what inferences can be made about the aching pain described, and to

what process can we attribute the phenomenon of "adaptation" to this pain?

Undoubtedly, the degree of pain experienced is closely allied to the strength of the cold stimulus, since the intensity, as well as the total amount of pain felt, increases in direct proportion to the lowering of the temperature of the water bath in which the hand is immersed. The first question to be answered is whether or not the pain thus experienced depends upon extreme stimulation of cold sensation (21, 22, 23, 24). Evidence upon this point is complete. As soon as the subject's hand is immersed he feels cold but no pain. The cold sensation continues throughout the cycle of pain and persists unaltered long after the pain is gone. In the experiment in which ischemia of the nerve was obtained by occluding the blood supply to the arm for 45 minutes, cold sensation

in the hand was abolished. "Cold pain", however, could be induced as usual. Clearly, the pain is not secondary to stimulation of the sense of cold.

Since separate pain fibers mediate the sensation of "cold pain" it is desirable to know their character. The experiments on partial block of the nerve trunks by ischemia, on the one hand, and procaine, on the other, suggest that the fibers in question are the small, non-myelinated ones of class C.

It is clear that purely local changes in the immersed part are responsible for initiating the pain, as evidenced from the distribution of the sensation as well as from the fact that its behavior is not altered by interruption of the blood supply and most of the nervous impulses going to the part. Furthermore, the purely local character of the phenomenon was demonstrated in the experiments in which parts were immersed successively into the water bath.

The fact that the intensity of pain experienced parallels closely the degree of cold, and the finding that "adaptation" is effective only while the hand and water temperature difference is small, have already been emphasized. These data suggest that the thermal gradient in the hand is responsible for "cold pain" and that, when the gradient becomes less after the first minute of cooling, "adaptation" occurs because the stimulus for pain is abolished. It seems unlikely that the site of production of this pain stimulus is in the skin since the character of the pain is not that of any other known cutaneous pains. These are invariably bright and burning in quality, while "cold pain" is deep and aching. The deeper tissues and blood vessels are the other possible sites where "cold pain" may be initiated.

Certain findings suggest that the blood vessels may be concerned in the production of "cold pain". There is a marked vasoconstriction associated with the occurrence of "cold pain" which is reflected in the tracings of the amplitude of pulsations of the digital artery. With the onset of "adaptation", there appears to be a relaxation of the vasospasm and an increase in the amplitude of the digital pulse toward the control level.

Several investigators have studied blood vessels with reference to their pain sensitivity (25, 26, 27, 28). The experiments on humans by Ray and

Wolff (20) show clearly that cranial arteries are pain-sensitive to faradic and mechanical stimulation. If the vessels were stretched, distended from within or without, pinched or pulled, very definite pain resulted.

If pain results from vasoconstriction, then it must be a purely local phenomenon. What is the relationship of the reflex vasomotor activity with its resultant hypertension, however, to the other events which occur as a result of plunging a hand into cold water? Could it represent simply a reaction to the pain experience? It is known that pain applied anywhere on the body induces hypertension and, as we have emphasized repeatedly, the height of blood pressure rise obtained corresponds closely to the intensity of pain experienced, which in turn depends upon the degree of cooling. Hines and Brown (1) separate subjects into three groups according to their blood pressure rise in the "cold pressor" test. They name the categories—Hyperreactors, Normal Reactors, and Hyporeactors. They look upon the Hyperreactors as potential hypertensives. Whether or not this inference is justifiable, it is quite clear that subjects do react differently to local cooling in terms of blood pressure rise. Moreover, they react according to a definite pattern. In a given individual, whether a hyper-, hypo-, or normal reactor, we found that the level to which the blood pressure rose increased in proportion to the lowering of the temperature of the water bath (Figure 8).

The vasopressor effect seems clearly to be influenced by the pain. In the experiment in which all the other sensory modalities were blocked by ischemia of the nerve, the elevation of blood pressure occurred as usual with "cold pain". Analgesic drugs of various sorts—*aspirin*, *codeine* and *alcohol*—lowered the pressor effect as they reduced the pain. In the subject who had had a recent cervical sympathectomy on the right, we found that much more intense pain was experienced when the sympathectomized hand was plunged into the cold water than when the normal hand was immersed. In other respects, sensory examination of the two hands was identical. A much greater blood pressure elevation was associated with the more painful experience, however.

It appears that the pressor effect in these experiments is related either to the pain itself or to

the subject's reaction to the pain. Several facts indicate that the latter possibility is the more likely. Schumacher and others (29) have shown that, while reaction to pain is by no means constant from one individual to another, the pain threshold of a large group of individuals is practically identical. This suggests but does not prove that the intensity of "cold pain" experienced by all subjects is about the same. The differences in blood pressure, then, must be due to differences in reaction to the pain from one subject to another. Some confirmation of this notion was obtained from an experiment in which a Hyperreactor was given 0.26 gram of sodium pentobarbital hypodermically in divided doses one hour apart. One-half hour after the second dose his hand was placed in water at 0° C. and his blood pressure response was that of a Hyporeactor. It has been shown that barbiturates in therapeutic dosage have relatively little effect on the pain threshold. The change must have been due to the effect of the drug on the subject's reaction to pain. Therefore, the term "Hyperreactor" of Hines and Brown appears to be a particularly appropriate one. The fact that this group reacts in such a way to a distressing stimulus which is equal in intensity to that to which others are exposed who react less strongly may well shed light upon this subsequent development of a permanent hypertension. These findings seem to support the thesis of Hines and Brown (1).<sup>1</sup> Whether or not the blood vessels are concerned in the mechanism of "cold pain" cannot be decided from the evidence in hand. There is, however, much to suggest that they are.

#### SUMMARY

A deep, aching, painful sensation induced by immersing a part of the body in cold water is described. The behavior of this phenomenon is found to follow a definite pattern, namely, that regardless of strength of stimulus, pain reaches its maximum in approximately 60 seconds. Thereafter, the pain gradually subsides, giving way to

a sensation of "pins and needles" which soon, in turn, is terminated. The intensity of the pain and the total amount of pain were found to depend directly upon the degree of cooling, with "adaptation" occurring when the difference between the hand and water temperature was small. Warming the hand or lowering the bath temperature abolished the protective effect of "adaptation". The mechanism whereby the pain is produced and the reason for its "adaptation" were investigated. It was found that:

1. This pain is entirely separate from and independent of the sensation of cold itself.

2. This type of pain does not show the phenomenon of spatial summation since exposure of one finger to cold causes pain of equal intensity to that experienced when the whole hand is so exposed.

3. The pain may be induced on nearly all parts of the body. In each case the highest bath temperature at which pain can be obtained is 18° C.

4. The skin temperature of the immersed part decreases rapidly for the first minute after immersion and then much more slowly.

5. The amplitude of pulsation of the digital artery parallels inversely the intensity of "cold pain" experienced.

6. The blood pressure-raising effect is proportional both to the intensity of pain experienced and to the degree of cold.

7. Ischemia, which is known to produce pain in muscular structures, is not a significant factor in the production of "cold pain".

8. By selective partial block of nerve trunks, it was indicated that "cold pain" is carried by the small, non-myelinated fibers of class C.

9. Sympathectomy was found to augment the intensity of pain derived from cold.

10. It was found possible to lower the bath temperature to zero without pain being felt in the immersed hand if the cooling were carried out slowly enough.

11. By successively cooling different parts it was learned that the production of "cold pain" depends on local changes in the part cooled.

12. Evidence is presented which indicates that the elevation of blood pressure which results from immersion of a part in cold water is a measure of the subject's reaction to "cold pain".

<sup>1</sup> There is one important modification which should be introduced into the technic of the "cold pressor" test. If the thermal stimulus is to be compared from one test to another, it is essential that the water in which the hand is immersed be stirred vigorously in order to avoid a warm zone in the immediate vicinity of the immersed hand.

## CONCLUSIONS

Pain due to local cooling is altogether separate from the sensation of cold itself. It is apparently mediated through small, non-myelinated fibers of class C. Its intensity, however, depends directly upon the degree of cooling. The stimulus required for the production of "cold pain" may be found in the thermal gradient in the tissues of the immersed hand. It is possible that this stimulus brings about a painful vasospasm in the part. Relaxation of this local vasospasm may occur as the thermal gradient is decreased, thus accounting for "adaptation". It appears that the "cold pressor" effect is a measure of reaction to pain.

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# FORMULAE FOR AFFERENT AND EFFERENT ARTERIOLAR RESISTANCE IN THE HUMAN KIDNEY: AN APPLICATION TO THE EFFECTS OF SPINAL ANESTHESIA

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Glomerular dynamics are steadily becoming more comprehensible as the trenchant methods for studying renal physiology developed by Homer Smith and his coworkers (1, 2) are further extended. Their recent analysis of the problem, using the current conception of the glomerulus as a pure ultra-filter operated by the hydrostatic pressure of the blood, is based on various presumptions—some of them explicit and supported by evidence, others tacit or not supported by evidence (2).

An examination of these basic questions leads to formulae, different in certain respects from those developed by these workers, which can be applied clinically to determine the actual afferent and efferent arteriolar resistance of human kidneys. We shall first present these formulae, with an example of a clinical application, before proceeding much further with their detailed derivation.

Fundamentally, the essence of a quantitative description of resistance, whether applied to flow of fluid or electricity, is the representation of resistance in terms of flow and pressure, the customarily measured quantities. In electricity, Ohm's law defines resistance as the ratio of the potential drop, in volts, along the resistor, to the electric current flow in it, in amperes. In hydraulics, Poiseuille, who was brought to the problem by his interest in the resistance of the blood vessels to the circulation of the blood, has similarly defined resistance as the ratio of the fall in pressure from one end to the other of a continuous wetted tube, to the rate of flow through it, after being multiplied by the viscosity of the fluid. Thus:

$$\text{Resistance} = \frac{\text{drop in pressure}}{\text{rate of flow} \times \text{viscosity}}.$$

It is, of course, applicable in the simple form only if certain conditions are fulfilled. For ex-

ample, it is presumed that the flow into the resistance is the same as the outflow: there are no leaks. In the discussion of the derivation of our formulae, the matter of the applicability and modification of Poiseuille's law and the validity of the choice of the points along the kidney's vascular system between which resistance is to be measured will be of importance. Approximations for the changes in viscosity of the blood will also be offered and supported. For this purpose, empirical formulae are to be derived which will relate the viscosity of plasma and whole blood to the serum protein content and hematocrit.

By itself, Poiseuille's law may not be directly applied to renal afferent and efferent arteriolar resistance. One must add the hypothesis, well supported by Homer Smith *et al.* (2), and which represents currently accepted views on the kidney's function, that in the glomerular capillaries the pressure of the blood filters out non-protein fluid into Bowman's capsule until finally the opposing osmotic force of the blood proteins thereby concentrated rises to equal it. This fluid then becomes the raw material from which the kidney's tubules elaborate urine. In the glomerulus, the filtrate is osmotically in equilibrium, across the capillary membrane, with the blood as it starts to enter the efferent arteriole.

This hypothesis of osmotic equilibrium, like Poiseuille's law, is subject to quantitation. Knowing the degree of increased concentration of the blood proteins produced by glomerular filtration, we can estimate from an empirical equation the resulting increased osmotic pressure and, therefore, the blood pressure in the terminal portion of the glomerular capillaries. In this way, we obtain one of the pressure measurements needed to apply Poiseuille's law to the kidney.

The rates of flow of blood and of glomerular

filtration are determined, clinically, by means of the diodrast and inulin clearances (1).

## PART I

### Formulae

The final formulae developed require definitions of certain symbols. We shall give them here briefly; they are more exactly defined in Part II.

Let  $R_A$  = resistance of afferent arterioles in mm. Hg per cc. per minute.

$R_E$  = resistance of efferent arterioles in mm. Hg per cc. per minute.

$R = R_A + R_E$ , total resistance of renal arterioles in mm. Hg per cc. per minute.

$I$  = inulin clearance in cc. per minute.

$D$  = diodrast clearance in cc. per minute.

$F = \frac{I}{D}$ , the filtration fraction.

$S$  = grams of protein in 100 cc. serum.

$H_c$  = hematocrit, as a fraction of 1.

$H = \frac{I}{1 - H_c}$ .

$k$  = a constant dependent on the hematocrit. For all, except grossly abnormal hematocrits,  $k = 0.47$ .

$P_M$  = mean of systolic and diastolic brachial blood pressures in mm. Hg of the recumbent subject.

Where

$$P_o = \frac{2.34S}{1 - 0.0542S} \quad \text{and} \quad P_{o'} = \frac{2.34S}{1 - 0.0542S - F}$$

$$R_A = \frac{P_M - P_{o'} - 40}{HD}$$

$$R_E = \frac{(1 - kF)(P_{o'} - P_o + 10)}{HD}$$

or

$$R_E = \frac{(1 - 0.47F)(P_{o'} - P_o + 10)}{HD}$$

For normal subjects, such as those reported by Smith and his coworkers (3), the formulae can be simplified. Using the average hematocrit of 43 per cent<sup>1</sup>; serum protein as 7 grams per 100 cc., with an osmotic pressure of the corresponding plasma of 26.4 mm. Hg, the formulae become

$$R_A = \frac{P_M - P_{o'} - 40}{1.755D}; \quad R_E = \frac{(1 - 0.47F)(P_{o'} - 16.4)}{1.755D}$$

### Clinical application

We shall now proceed to exemplify the use of these formulae in clinical material. There has been considerable discussion as to the part man's renal nerves play in controlling blood flow through the kidneys. On the assumption that spinal anesthesia abolishes any possible neuro-

genic control of the kidneys, Smith and his collaborators (3) have examined the changes produced in diodrast and inulin clearances and in blood pressure in a series of normal, basal, supine subjects. They have reached the conclusion that the kidneys are "not dependent upon tonic activity of the central nervous system."

Examining their cases, as reported, we omit, as does Smith, any correction factor for diodrast clearance. We use the set of simpler formulae above, as individual hematocrit and serum protein are not recorded. The results of this application are given in Table I.

The average fall in renal arteriolar resistance is found to be 32 per cent with a probable error of 4.2 per cent. Although so large a change may be due solely to autonomous activity of the denervated kidneys, it has not been demonstrated that abolition of central nervous influence is not involved. An analysis of data on blood pressure and cardiac output before and during spinal anesthesia in 11 cases, using the Poiseuille law formula with 20 mm. correction subtracted from mean blood pressure,<sup>2</sup> shows no consistent change in total body vascular resistance (4, 5). Consequently, a 32 per cent fall in renal arteriolar resistance would appear significant.

The afferent arterioles always dilated; the average is 70 per cent fall in resistance.<sup>3</sup> The afferent arteriolar resistance always fell more than the efferent arteriolar resistance. The efferent arteriolar resistance fell in 5 of 7 cases. In the last case, with a 50 per cent increase in efferent arteriolar resistance (if there is no technical failure) one would suspect either autonomous constriction of the efferent arteriole or a response to an increase in circulating adrenalin elicited by the fall of mean blood pressure to 75 mm. Hg. (Normally, in the unanesthetized subject both arterioles appear to be active (6).)

<sup>2</sup> See Part II, *The actual application of Poiseuille's law.*

<sup>3</sup> In 2 cases under spinal anesthesia with extremely abnormal blood pressures of 64 and 75 mm. Hg, the formula for afferent resistance breaks down, becoming negative. This would indicate that in this circumstance intracapsular and intrarenal pressures are less than the values we have used as normal. A modification of these quantities so that the intracapsular urinary perfusion pressure remains 10 mm. Hg would leave efferent arteriolar resistance unchanged, while increasing afferent arteriolar resistance and, to a much lesser extent, total arteriolar resistance.

<sup>1</sup> Dr. Homer Smith has very kindly supplied this datum.

TABLE I  
Effects of spinal anesthesia on renal arteriolar resistance\*

Subject number	Condition	Clearances		HD Renal blood flow	PM Mean blood pressure	PG Glo- merular intra- capillary pressure	RA Resist- ance of afferent arterioles	RE Resist- ance of efferent arterioles	R Total arte- riolar resist- ance	RA/RE Ratio of afferent to efferent resist- ance	$\Delta RA/RA$ Change in afferent arte- riolar resist- ance	$\Delta RE/RE$ Change in efferent arte- riolar resist- ance	$\Delta R/R$ Change in total arte- riolar resist- ance
		I Inulin (Glo- mer- ular f filtra- tion)	D Dio- drast (Plasma flow)										
		cc. per minute	cc. per minute	cc. per minute	mm. Hg	mm. Hg	$\times 1000$ mm. Hg per cc. per minute	$\times 1000$ mm. Hg per cc. per minute	$\times 1000$ mm. Hg per cc. per minute		per cent	per cent	per cent
18	Control	81	421	739	100	57.4	30.5	25.9	56.4	1.18			
18	Spinal	84	455	798	90	56.9	16.5	23.3	39.8	0.71	-46	-10	-29
13	Control	139	607	1065	100	60.9	17.9	20.5	38.4	0.87			
13	Spinal	128	655	1150	80	57.7	2.0	16.8	18.8	0.12	-89	-18	-51
11	Control	128	893	1567	94	53.7	13.0	10.3	23.3	1.26			
11	Spinal	116	910	1597	80	52.6	4.6	9.5	14.1	0.48	-65	-8	-39
20	Control	176	812	1425	100	59.7	14.2	14.7	28.9	0.97			
20	Spinal	155	771	1353	83	58.2	3.5	14.6	18.1	0.24	-75	-1	-37
17	Control	152	648	1137	95	61.5	11.9	19.7	31.6	0.60			
17	Spinal	102	596	1046	64	55.7		17.0	17.0	0.00	-100	-14	-46
12	Control	99	468	821	110	59.7	37.5	25.0	62.5	1.50			
12	Spinal	80	426	747	100	57.1	30.6	25.2	55.8	1.21	-18	+1	-11
10	Control	146	897	1574	86	55.1	6.9	11.0	17.9	0.63			
10	Spinal	128	664	1165	75	57.5		16.5	16.5	0.00	-100	+50	-8

\* See footnote 3 in text.

If we compute the average of the ratios to mean blood pressure of the blood pressure in the terminal portion of the glomerular capillaries ( $P_G = P_O + 20$ , see Part II) in the cases during anesthesia (renal denervation), we find a ratio of 0.70. If the 2 cases with abnormally low blood pressures are omitted, the ratio is 0.66. Winton and his collaborators report an average value of 0.66 (7) in the dog's isolated (denervated) kidney.

## PART II

### FORMULAE FOR GLOMERULO-DYNAMICS

#### Glomerular equilibrium

Smith *et al.* (2) first consider the relationship between the forces acting in the glomerulus. They reach the conclusion that, even under the condition of greatest renal blood flow, equilibrium exists between the difference in hydrostatic pressures on both sides of the glomerular membrane and the osmotic pressure of the concentrated blood before it leaves the glomerulus.

#### The change in hematocrit during glomerular filtration

Using the methods of Van Slyke (8), it can be shown that even during maximal glomerular hemoconcentration, when the plasma proteins

are concentrated 33 per cent (2), the resulting change in pH of the plasma (neglecting the buffering action of the red cells) is less than 0.006, which is less than the change occurring in the lungs. The hematocrit, with so small a shift, changes less than 1 per cent (9, 10). Consequently, in considering the effect of glomerular filtration on the red cells, the change in pH can reasonably be neglected.

We shall turn to the effect of the increase in plasma protein concentration on the red cells. While this increase is quite significant with respect to the glomerular membrane, which is permeable to all the crystalloid ions, it becomes insignificant so far as the red cell is concerned.

Recent studies using radioactive salts (11) have confirmed the previous finding that, in man, the red cell is, at least for a few hours, impermeable to sodium and potassium. The determination of the forces acting on the red cell membrane must, therefore, include the oncotic action of the impermeable crystalloid ions (sodium and potassium), as well as the protein, on both sides of the red cell surface.<sup>4</sup> On this basis, a rough compu-

<sup>4</sup> We are very much indebted to Dr. Homer W. Smith for calling this fact and its implication to our attention.



tation shows that plasma proteins account for only 1 per cent of the oncotic pressure of the plasma with respect to the red cell. Since the crystalloid ions permeate the glomerular membrane, they are not concentrated in the glomeruli and an increase of 33 per cent in plasma protein will produce a change of less than  $\frac{1}{3}$  per cent in the oncotic pressure acting on the red cells. Such a change is, of course, trivial so that we are justified in concluding that little, if any, water leaves the red cells during glomerular filtration and that their absolute volume remains fixed. A formula based on this premise is easily derived and is presented later when the change in viscosity of the blood during its passage through the kidney is considered.

#### *Osmotic pressure of concentrated blood*

Since the red cells are not osmotically active in the ultrafiltration occurring in the glomerulus, they can be excluded while this problem is examined.

To apply the results of *in vitro* determination of the osmotic pressure of blood to the kidney, one must be sure that the glomerular membrane acts like the artificial membranes in use. There appears to be little difference among the artificial membranes (12) and a suggested electrical charge on the glomerular membrane (13) seems unlikely and unimportant (14, 15). The observations on human transudates by Loeb, Atchley, and Palmer (16) under *in vitro* conditions showed the similarity between the living and artificial semi-permeable membrane and it therefore appears very likely that the results of laboratory determinations of osmotic pressure can be applied to glomerular ultra-filtration.

Excellent osmotic pressure measurements of blood have been made with an instrument invented by Hepp (10, 17, 18). It has not, as yet, been applied to human blood or blood more concentrated than normal—the range we are concerned with—so that, in determining the relationship between osmotic pressure and protein concentration in human plasma, we shall use the recent results of Adair and his coworkers (19). They have studied the osmotic pressure of fresh and redissolved dehydrated human serum up to, but not beyond, normal concentration and have given an empirical formula. At the maximum

glomerular hemoconcentration of 33 per cent, the osmotic pressure is found to be increased 65 per cent, so that the assumption by Smith *et al.* (2) of proportionality between osmotic pressure and protein concentration can lead to considerable error (20 per cent).

The Adairs' and Greaves' (19) formula, modified for 37.5° C., is:  $P_{O'} = \frac{2.34S'}{1 - 0.0543S'} + 0.1$ , where  $P_{O'}$  is the osmotic pressure in mm. of Hg of one source of normal human *plasma* and  $S'$  is the concentration of protein in grams per 100 cc. solution of the corresponding *serum*. The added number, 0.1, is derived from Hepp's work on cow's blood. The osmotic pressure of plasma is about 13 mm. H<sub>2</sub>O less than that of the corresponding serum (17). But the osmotic pressure at pH 7.4 (arterial blood) is about 14½ mm. of H<sub>2</sub>O greater than at pH 6.9, the value studied by Adair. The difference, 1½ mm. H<sub>2</sub>O, is 0.1 mm. Hg. Since this is less than  $\frac{1}{2}$  per cent of  $P_{O'}$ , it will be neglected. We then have:

$$(1) \quad P_{O'} = \frac{2.34 S'}{1 - 0.0542 S'}$$

This formula is to be used to obtain the osmotic pressure of plasma at any degree of hemoconcentration.

#### *Intracapsular and intrarenal pressure*

In the glomerulus, let us call the hydrostatic pressure of the blood, when it reaches osmotic equilibrium with its filtrate,  $P_G$ , expressed in mm. Hg, and correspondingly let  $P_C$  be the intracapsular pressure, the pressure against which the filtrate is formed. Smith *et al.*'s (2) description of equilibrium in the terminal capillaries of the glomerulus gives the equality:

$$P_G = P_{O'} + P_C.$$

Is the intracapsular pressure the same as intrarenal pressure, which, in the dog, has been estimated to average 10 mm. Hg and to be independent of arterial pressure (20, 21, 22)? Smith and his coworkers, in their analysis of the blood flow in and out of the glomerulus, assume that  $P_C = P_R$ . In such a circumstance, glomerular filtrate might conceivably be perfused through the tubules up to the loop of Henle where it be-

comes osmotically concentrated (1), in part by diffusion from a high to a low concentration of water. But it is doubtful that this would be at all a potent factor, and certainly its effect would be reversed during the secretion of hypotonic urine found at times in all mammals (1). Another possibility is the suction effect of the absorption of about 99 per cent of the filtrate (1) by the time the urine has reached the first part of the distal tubule. Its result would be to make a suction pump out of the proximal tubules, loop of Henle, and part of the distal tubules, the energy coming from the tubular epithelium. An objection to this notion is that the pump would have no valve; it would similarly suck urine backwards, up the collecting tubules, from the pelvis of the kidney. Neither of these hypotheses offers a force to move the concentrated urine through the last part of the distal and the collecting tubules. That the arterial pulsations of renal tissue milk urine towards the calices because the resistance of the remote retrograde branches of the system is greater than that of the central trunks seems a minor likelihood, incapable of effectively explaining the perfusion of urine through distal and collecting tubules. We are forced to the conclusion that  $P_C > P_R$ ; intracapsular pressure is greater than interstitial renal pressure.

O'Connor (23) calculated the glomerular filtrate perfusion pressure in the rabbit ( $P_C - P_R$ ) to be a minimum of 10 mm. Hg if the bore of the tubules were  $10 \mu$ . In view of the fact that "histological sections would indicate a rather higher value" for the lumen (24), and because we have noted other factors helping in perfusion, we should choose this minimum value for human filtrate perfusion pressure:  $P_C - P_R = 10$ . With Winton's average for interstitial pressure ( $P_R = 10$  mm. Hg), we have:  $P_C = 20$  mm. Hg; and  $P_O = P_O' + 20$ .

#### Poiseuille's law

Ideally, the resistance of a fixed tubular system to the flow of liquid is a constant defined by the ratio of the pressure difference used to perfuse it to the product of the rate of flow thereby produced and the relative viscosity of the fluid with respect to some standard. Inflow and outflow at the points of pressure measurement

must be equal for the definition of resistance to apply.

$$\text{Resistance} = \frac{\text{Perfusion pressure}}{\text{rate of flow} \times \text{relative viscosity}}.$$

Experimental *in vivo* confirmation of this relationship, somewhat modified for the kidney, is crude (21). Perfusion experiments have shown that, while it applies with modification (a constant is subtracted from pressure) to the isolated hind-limb of the dog (25), it does not apply to the isolated dog's kidney (26). However, the isolated dog's kidney behaves quite differently, in certain respects, from the kidney in the anesthetized or unanesthetized dog. Its urine is hypotonic; its inulin clearance (glomerular filtration rate) is quite considerably lower (more than 50 per cent); its creatinine clearance is not uniformly proportional to inulin clearance (27). While, in man, inulin clearance is approximately independent of renal blood flow (2), in the isolated or anesthetized dog's kidney, this is not the case (27). The mechanism of dilution diuresis also appears to differ in the isolated and anesthetized kidney (28).

The presumption attending the application of Poiseuille's law to an isolated organ is that its resistance remains constant while the flow and the perfusion pressure which cause it vary. If the isolated organ's arterioles respond autonomously to the change in pressure, and the denervated anesthetized kidney, unlike the extremities, appears to do so (29, 30), this presumption would be false. It does not seem unreasonable, therefore, to apply Poiseuille's law to man's intact kidney. Smith and his co-workers have done so, without the modification found necessary in the hind-limb (25), analyzing separately afferent and efferent arteriolar resistance. We shall proceed with this analysis.

#### Resistance of afferent arterioles

Since the blood undergoes no change in viscosity before the glomerulus, no correction for viscosity is needed if only the afferent arteriolar resistance referred to normal blood is measured. However, the two points of pressure reference chosen by Smith *et al.* are average arterial pressure and hydrostatic blood pressure in the glomerulus when osmotic equilibrium with ultra-

filtrate is reached, which must be near the efferent arteriole.

We can consider afferent resistance defined in this way ( $R_A$ ) to be composed of true afferent arteriolar resistance, ending at Bowman's capsule ( $\bar{R}_A$ ) and the resistance of the glomerular capillaries ( $R_G$ ) to perfusion ( $R_P$ ) and filtration ( $R_F$ ). Thus, we would have:

$$R_A = \bar{R}_A + R_G.$$

Since the filtration and perfusion resistances of the capillaries are parallel, they are computed in the usual way:

$$\frac{1}{R_G} = \frac{1}{R_P} + \frac{1}{R_F}.$$

It is found that, at the mean basal plasma filtration fraction of 19 per cent (2), where the effect of changes in viscosity of perfused blood and filtered plasma (they are opposite in direction and tend to cancel) are neglected, the result of computing  $R_G$  with the above formula, using Poiseuille's law, is less than 11 per cent different from the result when we compute  $R_G$  as:

$$R_G = \frac{\text{fall in pressure in glomerular capillaries}}{\text{rate of flow of blood into glomerulus}}.$$

While an error of 11 per cent in  $R_G$  may seem considerable, it is relatively small in its effect on  $R_A$ , as defined above to include  $R_G$ , since  $R_G$  is small compared with  $\bar{R}_A$ ; the pressure drop in the glomerulus itself is small compared with that in the afferent arteriole.<sup>5</sup>

By the time the blood in the glomerular capillaries reaches the efferent end, its volume has been diminished by filtration and its viscosity has increased due to the increase in the hematocrit and the concentration of plasma protein. In Poiseuille's law, the denominator of the expression for resistance is the product of flow and viscosity. Since flow decreases and viscosity increases, the effects of the two errors in neglecting these changes are in opposite directions. Later, in the consideration of the efferent arteriolar resistance, it will be shown that these errors almost cancel each other. It is for this reason, as well as because  $R_G$  is small compared with

$\bar{R}_A$ , that we introduce little error in computing  $R_A$  up to the equilibrium point in the glomerular capillaries, near the efferent arterioles, without allowing separately for filtration resistance, reduced flow, and increased viscosity. Smith and his associates likewise make the same choice, including the glomerulus in afferent arteriolar resistance without special adjustment (2).

### *Resistance of efferent arterioles*

The problem of the efferent arteriole is different in that in it the blood viscosity is increased throughout the major fall of pressure, up to the point where the glomerular filtrate has been reabsorbed in the peritubular capillaries. Smith and his coworkers make no allowance for the increase in viscosity since they consider it negligible (2).

### *Effect of change in viscosity*

Let us compute the importance of this change to ascertain how large a factor it is.

It has been shown that the viscosity of blood depends on the hematocrit, but that it is also proportional to the viscosity of the plasma (32, 33). The maximum plasma protein concentration observed by Smith and his associates is 33 per cent (2), so we are interested in the range of plasma viscosity from 7 grams per cent (taken as normal) to 9.3 grams per cent. While there have been many attempts to offer general formulae relating the concentration of solute to viscosity, none has been completely successful. However, in our range, a simple linear relationship is an excellent empirical one (34, 35). Such a formula, based on the data for serum viscosity in the proper range (35), has been derived by the method of least squares, modified with an increase of 22½ per cent at normal hemoconcentration to obtain the viscosity of the corresponding plasma (36, 37). Where  $V'$  is relative viscosity of the corresponding plasma with reference to water as unity, and  $S'$  is defined as previously for osmotic pressure, we obtain:

$$(2) \quad V' = 0.60 + 0.204S'.$$

We must now compute the change in hematocrit caused by glomerular filtration.

Let  $H_e$  = hematocrit of blood before ultrafiltration.  
 $H_e'$  = hematocrit of blood after ultrafiltration.

<sup>5</sup> The change of kinetic into potential energy is negligible (31).

$D$  = diodrast clearance of plasma in cc. per minute corrected for incomplete renal extraction and residue in red cells (2) (rate of flow of plasma through kidney).

$I$  = inulin clearance in cc. per minute (2) (rate of glomerular filtration by the kidney).

$F = \frac{I}{D}$ , filtration fraction.

The new hematocrit is found:

$$H_c' = \frac{\text{unchanged volume of RBC}}{\text{volume of RBC} + \text{initial volume of plasma} \div \text{degree of increased plasma concentration}}.$$

Or,

$$H_c' = \frac{H_c}{H_c + (1 - H_c)(1 - F)}.$$

On this basis, with  $F$  at its maximum value of 0.33, and taking a high initial hematocrit of 60 per cent, we find that  $H_c'$  is 69 per cent. Consequently, we are interested in a range of hematocrit from about 35 to 70 per cent. The effect of hematocrit on the viscosity of blood in the dog has been carefully studied by Whittaker and Winton (25). Using their curve (p. 358) in this range, we have obtained an excellent fit with the following empirical equation:

$$U' = \frac{V'}{V} \left( 0.27 + \frac{0.983}{1 - H_c'} \right).$$

Here  $U'$  is the viscosity of whole blood of hematocrit  $H_c'$  and plasma viscosity  $V'$ , while  $U$  and  $V$  are the values for unconcentrated normal blood ( $H_c' = H_c$ ;  $V' = V$ ).

Formulae have been given for all the quantities determining  $U'$  so that we can compute the relative change caused by glomerular filtration. We use for the normal hematocrit ( $H_c$ ) Smith's average value of 43 per cent.<sup>1</sup>

The maximum increase in blood viscosity occurs when the filtration fraction is 33 per cent. In this case, with relative viscosity entering into the denominator of Poiseuille's law, efferent arteriolar resistance would be estimated at the most 46 per cent too high, if the increase in blood viscosity caused by glomerular filtration is neglected. But if the loss of glomerular filtrate is also disregarded in estimating efferent arteriolar flow, along with the increase in viscosity, the result is at the worst 118 per cent of the more exact value, an error of 18 per cent. We see,

then, that if an approximation is to be made, it is wiser to neglect both the reduction in flow and the increase in viscosity of the blood in the efferent arteriole, rather than either one alone, since they tend to compensate each other. At mean basal plasma filtration fraction (19 per cent), these errors are considerably reduced—23½ per cent and 10 per cent, respectively. This illustrates in numerical form our previous statement that it is proper to neglect the opposing effects on resistance of changes in viscosity and blood flow occurring in the glomerulus when defining afferent arteriolar resistance. Strikingly enough, we see that the error introduced by this approximation for the resistance of the efferent arteriole is nearly proportional to the filtration fraction. Thus  $0.47F$  can be applied to correct our simplified formula for efferent resistance where the hematocrit is properly taken as the average, 43 per cent. Other factors of  $F$  can be used for other values of the hematocrit, if need should arise. It may be noted that the more precise expression involving viscosity and reduced blood flow depends only on clinically measurable quantities. We have:

Efferent arteriolar resistance

$$= \frac{\text{pressure difference} \times (1 - 0.47F)}{\text{whole blood flow through kidney}}.$$

#### *The definition of efferent arteriolar resistance*

The next step in the definition of efferent arteriolar resistance, having considered the changed viscosity and the reduced flow in this arteriole, is to consider what the perfusion pressure is. Certainly the equilibrium pressure at the efferent end of the glomerular capillaries, used as the terminal one in the definition of afferent resistance, is the correct initial one and we shall follow Smith *et al.* (2) in using it, although computing its value, as above, somewhat differently. The ideal terminal pressure would be the one at the arterial end of the peritubular capillaries, before significant change in the rate of blood flow due to reabsorption of the glomerular filtrate has occurred. But this pressure is not known and is certainly a variable one, depending on rate of flow and other factors, so that a fixed estimate of its size is unwarranted. As the blood

passes through the capillaries to the venules,<sup>6</sup> its volume is restored to 99 per cent of its original value by the reabsorbed glomerular filtrate and its viscosity falls back to normal. This is roughly the reverse of the situation in the glomeruli. By choosing venous capillary pressure as our terminal pressure in the definition of efferent arteriolar pressure, we introduce an error, but once again the effects of falling viscosity and rising flow are in opposite directions; the fraction involved of the total efferent arteriolar resistance defined is small, and so the net effect can be neglected. Homer Smith and his coworkers (2) have similarly chosen for their terminal perfusion pressure the venous end of the capillaries where hydrostatic blood pressure is very likely to be approximately equal to the sum of intrarenal and normal osmotic pressures. We shall, therefore, chose  $P_R + P_O$ , where  $P_O$  is normal blood osmotic pressure in mm. Hg, for the terminal pressure in our definition of efferent arteriolar resistance.

#### Arterial pressure

In measuring the perfusion pressure of the afferent arteriole, we should naturally choose the pressure in the renal artery. Since this is pulsatile, the best approximation would be its integrated mean value. This value is not the average of systolic and diastolic blood pressure but diastolic blood pressure plus 44 per cent of the pulse pressure (38). The fall in this more precise mean, as obtained from subclavian and femoral arteries in man, is usually about 2 mm. of mercury. The average of systolic and diastolic pressure is about 3 mm. Hg too high (38). It is therefore clear that the average of systolic and diastolic blood pressure taken at the arm is a good enough measure of mean renal artery pressure.

#### The actual application of Poiseuille's law

Previously, we have mentioned Whittaker and Winton's (25) finding that Poiseuille's law applies to the hind-limb of the dog perfused with blood, but with modification. They find that the perfusion pressure plotted against the flow is a straight line with an intercept at about 20 mm. of

mercury. That is to say, Poiseuille's law for blood is really:

$$\text{Resistance} = \frac{\text{Perfusion pressure} - 20}{\text{Rate of flow}}.$$

This phenomenon appears to be an inherent one of blood itself (25). There is further confirmation of it in the intact animal (39). In applying Poiseuille's law to the afferent arteriole, we shall therefore use this 20 mm. Hg correction. But we shall not use it in the efferent arteriole where blood flows from capillaries back to capillaries, not from artery to capillaries, as in the afferent arteriole and in Whittaker and Winton's experiments.

We are now ready to utilize Smith and his coworkers' (2) analysis of renal dynamics as modified above.

Let  $P_M$  = Average of systolic and diastolic blood pressure measured at the arm in mm. Hg.

$D$  = Diodrast clearance of plasma in cc. per minute, etc., as noted previously.

$I$  = Inulin clearance of plasma in cc. per minute, as noted previously.

$H_c$  = Hematocrit, as a fraction of 1.

$H = \frac{1}{1 - H_c} = \frac{\text{whole blood volume}}{\text{plasma volume}}.$

$HD$  = Total blood flow in cc. per minute.

$F = \frac{I}{D}$ , plasma filtration fraction.

$R_A$  = Afferent arteriolar resistance, as defined.

$R_E$  = Efferent arteriolar resistance, as defined.

$R = R_A + R_E$ , total arteriolar resistance, as defined.

The resistance units will be mm. Hg per cc. per minute with the viscosity of the unconcentrated blood take as unity.

Other symbols have already been defined.

The perfusion pressure for the definition of afferent arteriolar resistance is the difference between mean renal artery pressure and the equilibrium blood pressure in Bowman's capsule. Thus:

$$(3) \quad R_A = \frac{P_M - P_G - 20}{HD}.$$

The perfusion pressure for  $R_E$  is  $P_G - (P_O + P_R)$ ; the flow is  $HD - I$ . We have:

$$(4) \quad R_E = \frac{P_G - P_O - P_R}{(HD - I) \frac{U'}{U}}.$$

<sup>6</sup> The cortical tissues are mainly nourished by glomerular filtrate on its way to the capillaries.

Substituting for  $P_G$  its equivalent,  $P_C + P_{O'}$ , we write:

$$(5) \quad R_E = \frac{P_{O'} - P_O + 10}{(HD - I) \frac{U'}{U}}.$$

We have justified the substitution, where  $k$  is a constant dependent on the hematocrit,

$$(HD - I) \frac{U'}{U} = \frac{HD}{1 - kF}.$$

Accordingly,

$$(6) \quad R_E = \frac{(1 - kF)(P_{O'} - P_O + 10)}{HD};$$

$$(7) \quad R_A = \frac{P_M - P_{O'} - 40}{HD}.$$

The formula for  $P_{O'}$  has already been given (see Equation (1)), and  $P_O$  is found from this formula when for  $S'$  we substitute  $S$ , the clinically observed value for unconcentrated serum protein. The degree of hemoconcentration being  $\frac{1}{1 - F}$ , we have  $S' = \frac{S}{1 - F}$ .

Substituting the formula for  $S'$  in that for  $P_{O'}$ , we find that:

$$(8) \quad P_{O'} = \frac{2.34S}{1 - 0.0542S - F}.$$

$P_O$  is the value of  $P_{O'}$  when  $F$  is set equal to zero.

Equations (6) and (7) for  $R_E$  and  $R_A$  are therefore seen to be completely determined, with the aid of Equation (8), by the clinically determinable entities: serum protein, hematocrit, inulin and diodrast clearances, and blood pressure.<sup>7</sup>

#### SUMMARY

The application of Poiseuille's law to the kidney has been discussed and formulae have been developed to measure clinically, in man, afferent and efferent arteriolar resistance. A practical application to available clinical data on the renal effect of spinal anesthesia (denervation) in normal man has also been offered. At present, while normal man may lack tonic central nervous control of renal blood flow, this does not

appear to have been demonstrated. The evidence also does not preclude autonomous control by the kidney of its blood supply.

Incidentally, empirical formulae for the viscosity of human plasma and whole blood have been derived from observations in the literature.

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<sup>7</sup> The method of computing  $k$  has already been given under *Effect of change in viscosity*. Usually,  $k = 0.47$  is adequate, as most hematocrits are near 43 per cent.

passes through the capillaries to the venules,<sup>6</sup> its volume is restored to 99 per cent of its original value by the reabsorbed glomerular filtrate and its viscosity falls back to normal. This is roughly the reverse of the situation in the glomeruli. By choosing venous capillary pressure as our terminal pressure in the definition of efferent arteriolar pressure, we introduce an error, but once again the effects of falling viscosity and rising flow are in opposite directions; the fraction involved of the total efferent arteriolar resistance defined is small, and so the net effect can be neglected. Homer Smith and his coworkers (2) have similarly chosen for their terminal perfusion pressure the venous end of the capillaries where hydrostatic blood pressure is very likely to be approximately equal to the sum of intrarenal and normal osmotic pressures. We shall, therefore, choose  $P_R + P_O$ , where  $P_O$  is normal blood osmotic pressure in mm. Hg, for the terminal pressure in our definition of efferent arteriolar resistance.

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This phenomenon appears to be an inherent one of blood itself (25). There is further confirmation of it in the intact animal (39). In applying Poiseuille's law to the afferent arteriole, we shall therefore use this 20 mm. Hg correction. But we shall not use it in the efferent arteriole where blood flows from capillaries back to capillaries, not from artery to capillaries, as in the afferent arteriole and in Whittaker and Winton's experiments.

We are now ready to utilize Smith and his coworkers' (2) analysis of renal dynamics as modified above.

Let  $P_M$  = Average of systolic and diastolic blood pressure measured at the arm in mm. Hg.

$D$  = Diodrast clearance of plasma in cc. per minute, etc., as noted previously.

$I$  = Inulin clearance of plasma in cc. per minute, as noted previously.

$H_c$  = Hematocrit, as a fraction of 1.

$$H = \frac{1}{1 - H_c} = \frac{\text{whole blood volume}}{\text{plasma volume}}.$$

$HD$  = Total blood flow in cc. per minute.

$$F = \frac{I}{D}, \text{ plasma filtration fraction.}$$

$R_A$  = Afferent arteriolar resistance, as defined.

$R_E$  = Efferent arteriolar resistance, as defined.

$R = R_A + R_E$ , total arteriolar resistance, as defined.

The resistance units will be mm. Hg per cc. per minute with the viscosity of the unconcentrated blood taken as unity.

Other symbols have already been defined.

The perfusion pressure for the definition of afferent arteriolar resistance is the difference between mean renal artery pressure and the equilibrium blood pressure in Bowman's capsule. Thus:

$$(3) \quad R_A = \frac{P_M - P_G - 20}{HD}.$$

The perfusion pressure for  $R_E$  is  $P_G - (P_O + P_R)$ ; the flow is  $HD - I$ . We have:

$$(4) \quad R_E = \frac{P_G - P_O - P_R}{(HD - I) \frac{U'}{U}}.$$

<sup>6</sup> The cortical tissues are mainly nourished by glomerular filtrate on its way to the capillaries.

Substituting for  $P_G$  its equivalent,  $P_C + P_{O'}$ , we write:

$$(5) \quad R_E = \frac{P_{O'} - P_O + 10}{(HD - I) \frac{U'}{U}}.$$

We have justified the substitution, where  $k$  is a constant dependent on the hematocrit,

$$(HD - I) \frac{U'}{U} = \frac{HD}{1 - kF}.$$

Accordingly,

$$(6) \quad R_E = \frac{(1 - kF)(P_{O'} - P_O + 10)}{HD};$$

$$(7) \quad R_A = \frac{P_M - P_{O'} - 40}{HD}.$$

The formula for  $P_{O'}$  has already been given (see Equation (1)), and  $P_O$  is found from this formula when for  $S'$  we substitute  $S$ , the clinically observed value for unconcentrated serum protein. The degree of hemoconcentration being  $\frac{1}{1 - F}$ , we have  $S' = \frac{S}{1 - F}$ .

Substituting the formula for  $S'$  in that for  $P_{O'}$ , we find that:

$$(8) \quad P_{O'} = \frac{2.34S}{1 - 0.0542S - F}.$$

$P_O$  is the value of  $P_{O'}$  when  $F$  is set equal to zero.

Equations (6) and (7) for  $R_E$  and  $R_A$  are therefore seen to be completely determined, with the aid of Equation (8), by the clinically determinable entities: serum protein, hematocrit, inulin and diodrast clearances, and blood pressure.<sup>7</sup>

#### SUMMARY

The application of Poiseuille's law to the kidney has been discussed and formulae have been developed to measure clinically, in man, afferent and efferent arteriolar resistance. A practical application to available clinical data on the renal effect of spinal anesthesia (denervation) in normal man has also been offered. At present, while normal man may lack tonic central nervous control of renal blood flow, this does not

appear to have been demonstrated. The evidence also does not preclude autonomous control by the kidney of its blood supply.

Incidentally, empirical formulae for the viscosity of human plasma and whole blood have been derived from observations in the literature.

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<sup>7</sup> The method of computing  $k$  has already been given under *Effect of change in viscosity*. Usually,  $k = 0.47$  is adequate, as most hematocrits are near 43 per cent.



HAROLD LAMPORT

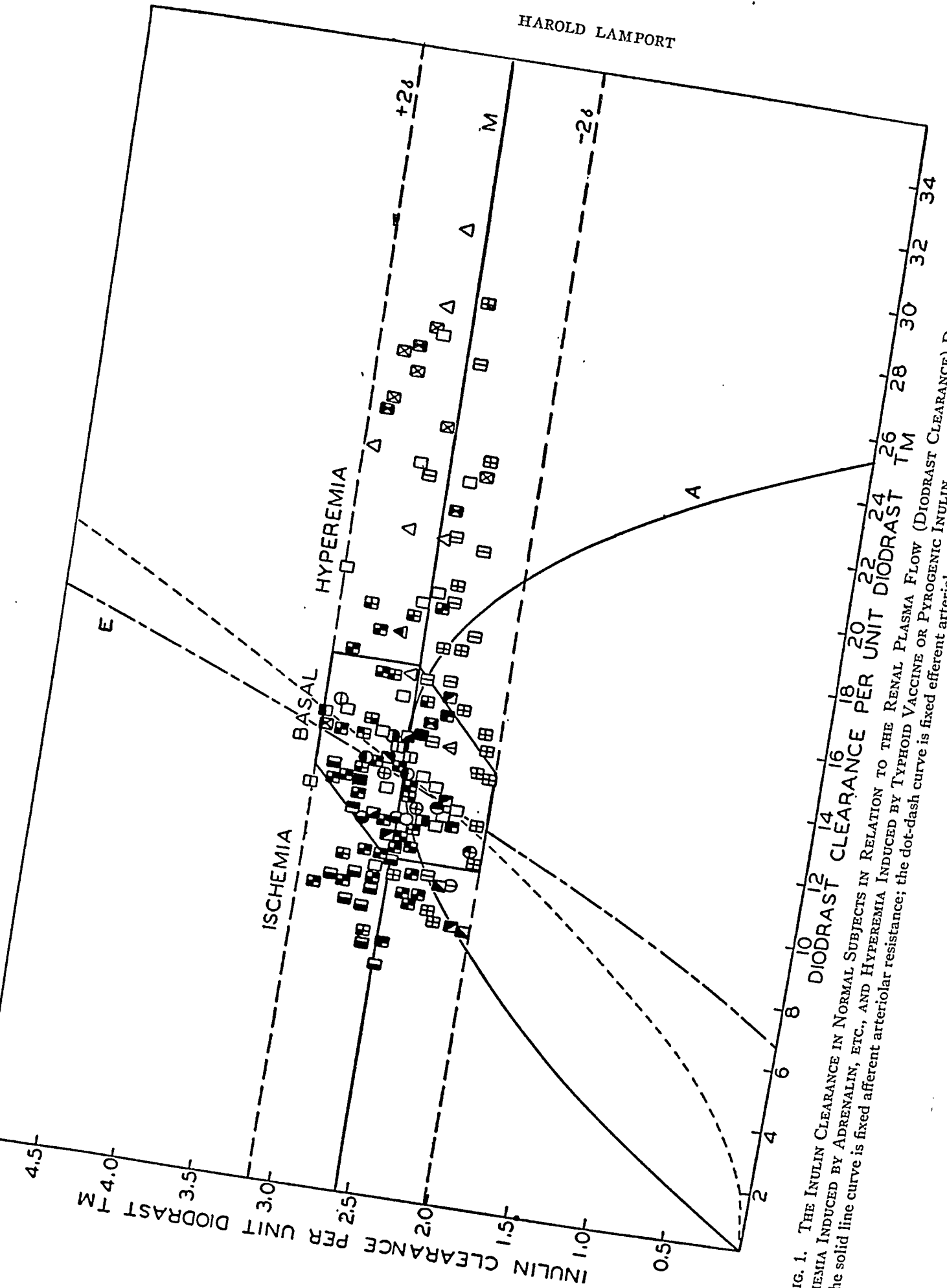


FIG. 1. THE INULIN CLEARANCE IN NORMAL SUBJECTS IN RELATION TO THE RENAL PLASMA FLOW (DIODRAST CLEARANCE) DURING BASAL CONDITIONS, ISCHEMIA INDUCED BY ADRENALIN, ETC., AND HYPEREMIA INDUCED BY TYPHOID VACCINE OR PYROGENIC INULIN. The solid line curve is fixed afferent arteriolar resistance; the dot-dash curve is fixed efferent arteriolar resistance (1)

- $P_o$  = osmotic pressure of unconcentrated blood in mm. Hg.  
 $P_{o'}$  = osmotic pressure of blood concentrated in glomerulus in mm. Hg.  
 $P_M$  = average of systolic and diastolic brachial blood pressures in mm. of Hg.  
 $S$  = concentration of protein in grams per 100 cc. of normal human serum.  
 $R_A$  = resistance of afferent arterioles (defined more precisely elsewhere) (2).  
 $R_E$  = resistance of efferent arterioles (2).  
 $k$  = constant, dependent on hematocrit, equal to 0.47 when hematocrit is 0.43.

As previously shown (2):

$$R_A = \frac{P_M - P_{o'} - 40}{HD};$$

$$R_E = \frac{(1 - kF)(P_{o'} - P_o + 10)}{HD};$$

$$P_{o'} = \frac{2.34S}{1 - 0.0542S - F}.$$

If we take  $S = 7.0$ ;  $H = 1.755$  (corresponds to hematocrit of 0.43);  $P_o = 26.4$ ;  $k = 0.47$ ; and the mean basal condition of  $D = 13.4$  and  $I = 2.56$ , as was previously done in a clinical application (2); and let  $P_M = 90$ , as have Smith and his collaborators,<sup>2</sup> we have:

$$D = 52.7 - \frac{17.3}{0.63 - F},$$

where  $R_A$  is constant at 0.540, the mean basal value found for  $D = 13.4$ ,  $I = 2.56$ . And, where  $R_E$  is fixed at 0.809, for the same values of  $D$  and  $I$ , the equation determining the resulting relationship of  $D$  and  $I$  is:

$$D = 11.55(1 - 0.47F) \left( \frac{1}{0.63 - F} - 1 \right).$$

While Figure 1 illustrates the fact that neither set of arterioles is fixed when renal blood flow varies, another way of demonstrating this is to compute, from the formulae, the effect on resistance of a 1 per cent decrease in renal blood flow or diodrast clearance, to which it is proportional, while the inulin clearance (glomerular filtration rate) stays constant. We find that such a change in flow at the mean basal point (1) requires approximately a 0.2 per cent decrease in afferent

arteriolar resistance and a 0.9 per cent increase in efferent arteriolar resistance.

Smith and his collaborators demonstrate that the consequence of assuming the fall in pressure in the efferent arterioles ( $P_E$ ) to be inversely proportional to the flow through them ( $Q$ ) is constancy of glomerular filtration rate, if their formulae are applicable (1). They consider that this inverse relationship is the result of fixed afferent arteriolar resistance. While we have offered evidence that this is not the case, let us determine the meaning of this relationship. In algebraic form, where  $W$  is a constant, it becomes:

$$P_E = W/Q; \quad \text{or} \quad W = P_E Q.$$

This is the analogue of power loss in the form of heat in electricity: watts = volts  $\times$  amperes. The assumption made, therefore, is really that the efferent arterioles vary in caliber so that the kinetic energy of the flowing blood dissipated in them as heat (friction loss) remains constant. The change in blood viscosity as a result of glomerular hemoconcentration must be considered negligible and osmotic pressure must be considered proportional to hemoconcentration for this relationship to be true (1).

Is it approximately true? If  $W$ , above, evaluated with regard to the effects of viscosity and non-linear osmotic pressure changes, remains fairly invariant, with changes in renal blood flow, the constancy of efferent arteriolar heat loss could be a good approximation. For  $Q$ , we use the flow modified to include the effects of viscosity, as justified elsewhere (2), and the formula for  $P_{o'}$  has been given. We then have:

$$W = (P_{o'} - P_o + 10) \left( \frac{HD}{1 - kF} \right).$$

Using the normal values, as before:

$$W = (P_{o'} - 16.4) \frac{1.755 D}{1 - 0.47F}.$$

Evaluating  $W$  at two different ranges of blood flow, ischemia and hyperemia, by letting  $I$  be constant at its mean value of 2.56 cc. and letting  $D = 7$  and 20 cc. per minute per unit diodrast tubular mass, we determine the change in  $W$  produced by a 1 per cent decrease in  $D$  (1 per cent

<sup>2</sup> An examination of the papers referred to shows that mean blood pressure does not change much during maximal renal ischemia due to adrenalin.

decrease in renal blood flow). We find that when  $D = 7$ , a 1 per cent decrease in  $D$  causes a 1.2 per cent increase in  $W$ , which is 442; when  $D = 20$ , a 1 per cent decrease in  $D$  produces an increase in  $W$  of 0.6 per cent from 258. It is therefore clear that the "constant"  $W$  varies considerably and the friction heat loss in the efferent arterioles is not approximately fixed.

#### SUMMARY

1. Formulae for the afferent and efferent arteriolar resistance to renal blood flow in man have been applied to available data for normal subjects under ischemic, basal, and hyperemic renal conditions.

2. It has been found that the resistance of both sets of arterioles vary simultaneously in maintaining constant glomerular filtration in the basal state and in adrenalin ischemia and pyrogenic hyperemia of the human kidney.

3. At the mean basal state, a 1 per cent decrease in renal blood flow per unit diodrast tubular mass is caused by approximately a 0.2 per cent decrease in afferent arteriolar resistance and a 0.9 per cent increase in efferent arteriolar resistance.

4. The heat loss due to friction of the blood flowing through the efferent arterioles is not approximately constant.

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# MECHANISMS OF RESPIRATORY FAILURE UNDER BARBITURATE ANESTHESIA (EVIPAL, PENTOTHAL)<sup>1</sup>

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With the growing use of the barbiturates in anesthesia and in medicine in general, it becomes increasingly important to understand the hazards this entails, not only so that accidents of the past may be explained but, chiefly, so that they can be prevented in the future. In this study our attention has been directed to the respiration. While other systems may also be affected adversely by the barbiturates, the respiration frequently gives the first warning of impending difficulties. Respiratory failure often plays an important part in death under these agents. This study was designed primarily to investigate the mechanisms leading to respiratory failure under evipal and pentothal anesthesia. Since the barbiturates are qualitatively very similar in their actions, it is probable that the mechanisms described here apply to many members of the group.

## EQUIPMENT AND PROCEDURES

*Animals.* Mongrel dogs weighing about 10 kilograms were employed. Blood pressure was recorded by means of a Ludwig manometer from the femoral artery. The representative material presented here was chosen from that obtained in experiments on 43 dogs under the two anesthetic agents considered.

*Anesthesia.* Evipal sodium [1 methyl 5 $\Delta$ ' cyclohexenyl 5 methyl barbiturate] in 10 per cent aqueous solution was administered intravenously in an initial dose of 50 mgm. per kgm. body weight. This was supplemented intravenously as needed during the preparation of the animal and the course of the experiment. Pentothal sodium [ethyl (1-methyl-butyl) thiobarbiturate] in 5 per cent aqueous solution was administered intravenously in an initial dose of 25 mgm. per kgm. body weight. Additional anesthetic was added intravenously as needed. The points illustrated hold true for both agents. Sometimes one agent, sometimes the other, is used to demonstrate a given point.

Depth of anesthesia was controlled as carefully as possible by observation of the state of a spinal reflex. The flexion reflex of the left semitendinous muscle was

evoked by electrical stimulation of the central end of the cut left sciatic nerve. The electrodes were chlorided silver wires flattened and perforated and stitched to the nerve. Insulation was insured with cotton packing around the electrodes and nerve. These electrodes were connected with the secondary coil of a Harvard inductorium. The primary was activated with a 1.5 volt dry cell. A hand-operated mercury contact key and a signal magnet were placed in circuit with this. The strength of the stimulating current was adjusted to give approximately a maximal response. The ipsilateral nerves to the hamstring muscles were left intact so that contractions of the divided semitendinous muscle might be recorded on the smoked drum. Essentially isometric recording was obtained by employing a tempered steel recording lever. The information as to depth of anesthesia obtained from the spinal reflex records was supplemented by frequent notes as to the state of the corneal and lid reflexes. Sluggish reflexes were designated as one plus and very active ones as two plus.

*Respiratory apparatus and recording.* Three simultaneous records were made of the respiration: The tidal excursions were recorded by means of a Hutchinson spirometer, specially constructed so as to allow an artificial increase or decrease in intrapulmonary pressure to be made during the recording (4); the intercostal and diaphragmatic respirations were recorded, as described by Gesell and Moyer (3), by means of paper bands which encircled the midthorax and the midabdomen of the torso from which the hair had been clipped; the segmental and combined respiratory responses were recorded on a smoked drum where an upstroke corresponds to inspiration. The animal was connected to an airway (where airflow was directed by two Tissot valves) and from this to the Hutchinson spirometer and a 60 liter steel tank, usually two-thirds filled with water. Interconnections through brass steam pressure valves permitted instantaneous shift to the gas mixtures contained in any one of three such steel tanks without interruption during recording. A soda lime cannister was inserted into the expiratory half-circuit of the system in such a way that the carbon dioxide would be removed during respiration except when the tank reserved for the administration of carbon dioxide was used.

*Gas analysis.* Blood gases were determined in duplicate by the method of Van Slyke and Neill. Oxygen content was determined in 1.0 cc. samples of heparinized whole blood taken under oil; carbon dioxide in 0.5 cc. serum.

At times mixtures of inspired gases were made up

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<sup>2</sup> Fellow of the National Research Council.

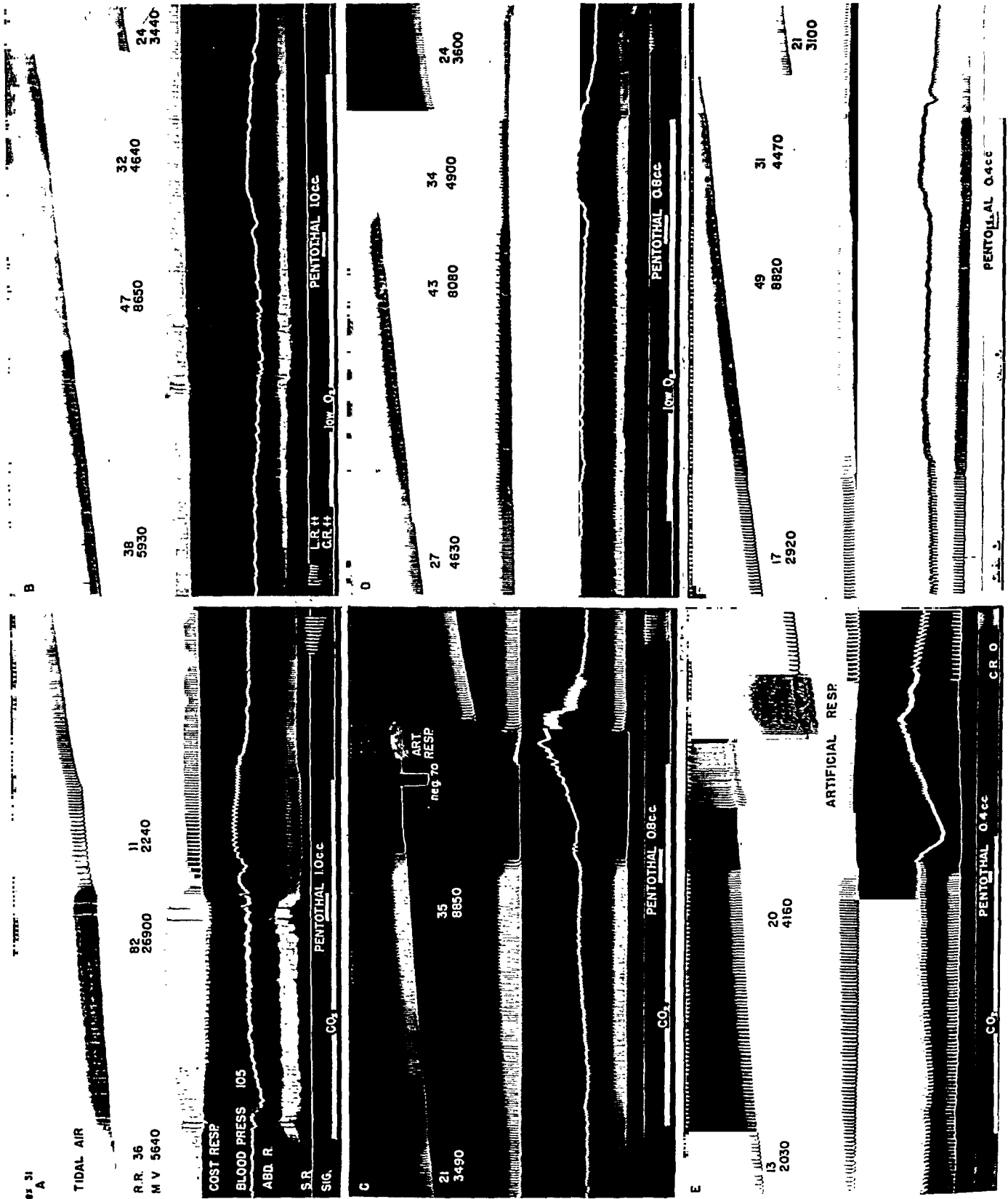


FIG. 1. PENTOTHAL (5 PER CENT SOLUTION) ANESTHESIA

In the records an upstroke corresponds to inspiration. Read from left to right. Time marker at 5-second intervals with minutes indicated. To avoid including otherwise uninteresting portions of records resulting from interference with respiration caused by sciatic stimulation, the reflex response records have been moved from nearby

parts of the record. Doses are totals administered at a given time in all cases. Except as specified, the animal breathed room air. Twelve per cent carbon dioxide was used. "Low oxygen" refers to 5 per cent oxygen. The base line for blood pressure is the signal line. Weight of dog 9.8 kgm.

specially. At other times the following mixtures were employed: In one tank 12 per cent carbon dioxide was mixed with 30 per cent oxygen and 58 per cent nitrogen. In another tank 9 per cent oxygen was mixed with 91 per cent nitrogen. The composition of the inspired gases was determined at intervals in the Henderson modification of Haldane's apparatus.

### RESULTS

Although the barbiturates have been in widespread clinical use for many years, and for a considerable number of years have been used for general anesthesia, it is a curious fact that relatively little attention has been given to a consideration of the conditions which enhance the toxicity of these agents; yet, the statement is almost axiomatic that a great hazard of the use of the barbiturates is their variability of action. Doubtless many still unknown factors are involved in this variability. Several conditions are encountered clinically which increase the toxicity of these agents: (1) gross overdosage followed by respiratory failure when the blood gases are normal, at least at first; (2) the respiratory response to the barbiturates when the blood oxygen content is low; (3) the respiratory response to the barbiturates when the blood oxygen content is high; (4) respiratory alteration and failure following administration of the drugs when the carbon dioxide content is high; (5) respiratory failure under a combination of (2) and (4) above; and, finally, (6) respiratory failure as a result of positive pressure in the airway and the breathing of 100 per cent oxygen. These conditions will be illustrated by representative examples taken from the 43 experiments.

(1) It hardly seems necessary to illustrate the production of respiratory failure by gross overdosage with barbiturates when the blood gases are initially within normal limits, for everyone who has employed these agents to any considerable extent experimentally or clinically has demonstrated this effect.

(2) The respiratory response to low oxygen is particularly interesting under the barbiturates. This has been discussed in detail by Moyer and Beecher (8, 9). For present purposes, this can be illustrated by Figure 1, B, D, F, and Figure 2. In Figure 1, Sections B, D, and F represent, respectively, light, moderately deep and deep barbiturate anesthesia. (Note the sciatic reflex evoked

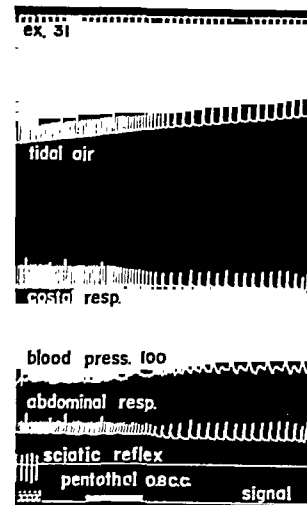


FIG. 1, D'. PENTOTHAL (5 PER CENT SOLUTION) ANESTHESIA

Details are as for Figure 1

by stimulation.) The responses of the pulmonary ventilation to four minutes of breathing 5 per cent oxygen, as well as the effects on ventilation of small doses of pentothal, are tabulated here:

TABLE I

Weight of dog: 9.8 kgm.; pentothal 5 per cent intravenously

Figure	Section	Depth of anesthesia	Pulmonary minute volume of ventilation		
			Initial	After 4 minutes on 5% O <sub>2</sub>	Immediately after additional 5% pentothal
1	B	Light	5930	8650	4640[1.0 cc.]
1	D	Moderately deep	4680	8080	4900[0.8 cc.]
1	F	Deep	2920	8820	4470[0.4 cc.]

These figures are presented for illustrative purposes only and are not to be construed as representing precise changes to be encountered on all occasions. These figures show, however, the general fact that, over a wide range of anesthesia, low oxygen in the inspired air effects a great increase in pulmonary ventilation. It is interesting to observe in passing that, although the initial minute volumes were progressively smaller with increasing depth of anesthesia, the low oxygen produced the same final effect, that is, the ventilation rose to the same level in each case, regardless of where it had started from. This will be considered in the

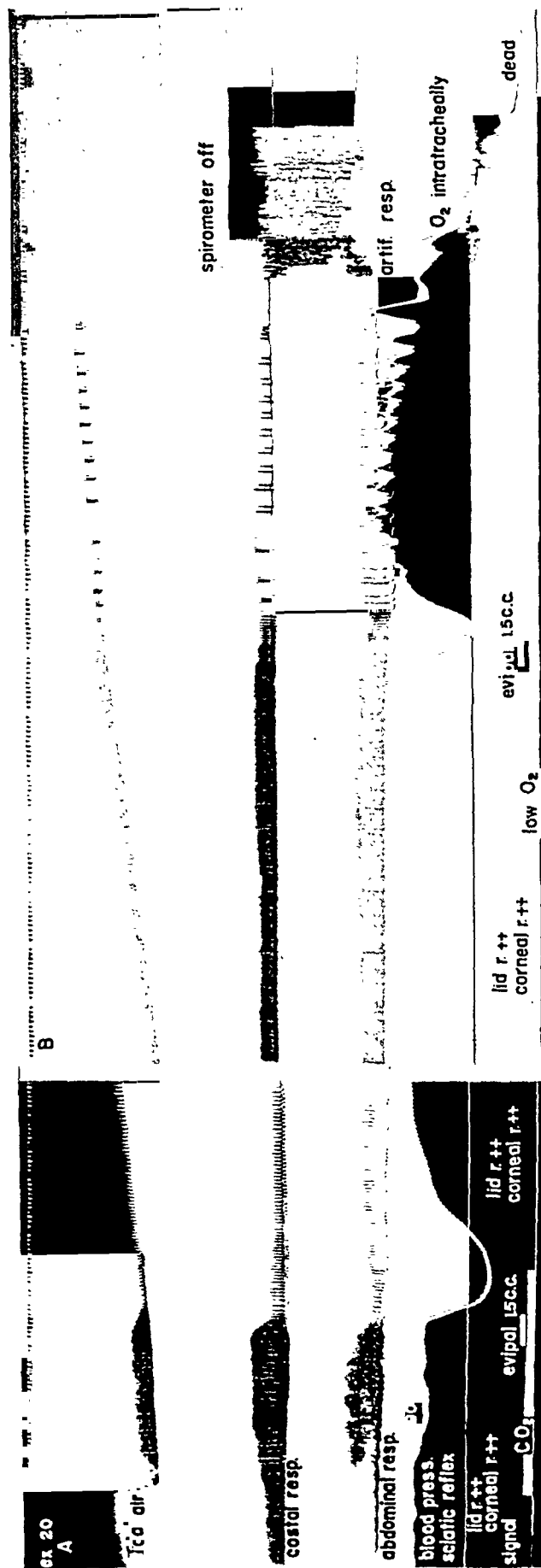


FIG. 2. EVIPAL (10 PER CENT SOLUTION) ANESTHESIA

Details are as for Figure 1. Weight of dog, 9.3 kgm. "Low oxygen" refers to 6 per cent

discussion. Table I contains further information of general importance: With increasing depth of anesthesia, smaller and smaller doses of drug will be necessary to produce a given reduction in ventilation in the presence of low oxygen stimulation. Under deep anesthesia, 40 per cent of the dose used in light anesthesia effected the same reduction in minute volume. (The initial ventilation under the deep anesthesia had not fallen lower than approximately 3000 cc. per minute; this ordinarily can be considered as adequate to keep the blood carbon dioxide level within normal limits, so it seems unlikely that any abnormal accumulation of carbon dioxide was present in the blood.) Notwithstanding the important stimulation of low oxygen, increasingly greater effects will be produced by given doses of barbiturate as the anesthesia deepens.

Inasmuch as a severe degree of oxygen shortage will of itself produce many of the signs of anesthesia, it is not surprising that a point will ultimately be reached where a dose of barbiturate that would not ordinarily have serious effect will, during low oxygen administration, produce a fatal result. This effect of low oxygen is illustrated in the lightly anesthetized animal whose record is shown in Figure 2, B. Evipal was the agent employed. Here, vigorous artificial respiration promptly instituted with high oxygen in the airway could not reverse the process, even though the heart continued to function for a considerable time after artificial respiration was instituted, as shown in the tracing. The earlier administration of the same dose, even in conjunction with a toxic concentration of carbon dioxide, had no serious effect (Figure 2, A). Sufficient time was allowed to elapse before the final barbiturate injection was made so that the animals were at essentially the same levels of anesthesia in Sections A and B. This, of course, must not be construed as indicating that, under all circumstances, the percentage of low oxygen employed here is more toxic than the carbon dioxide concentration used. In fact, Figure 1 nicely demonstrates that, under the circumstances of that experiment, the concentration of carbon dioxide used was more depressant than the 5 per cent oxygen with which it was compared. Figure 2 is of use in illustrating that a severe degree of oxygen shortage in conjunction with a not ordinarily dangerous dose of the barbiturate can

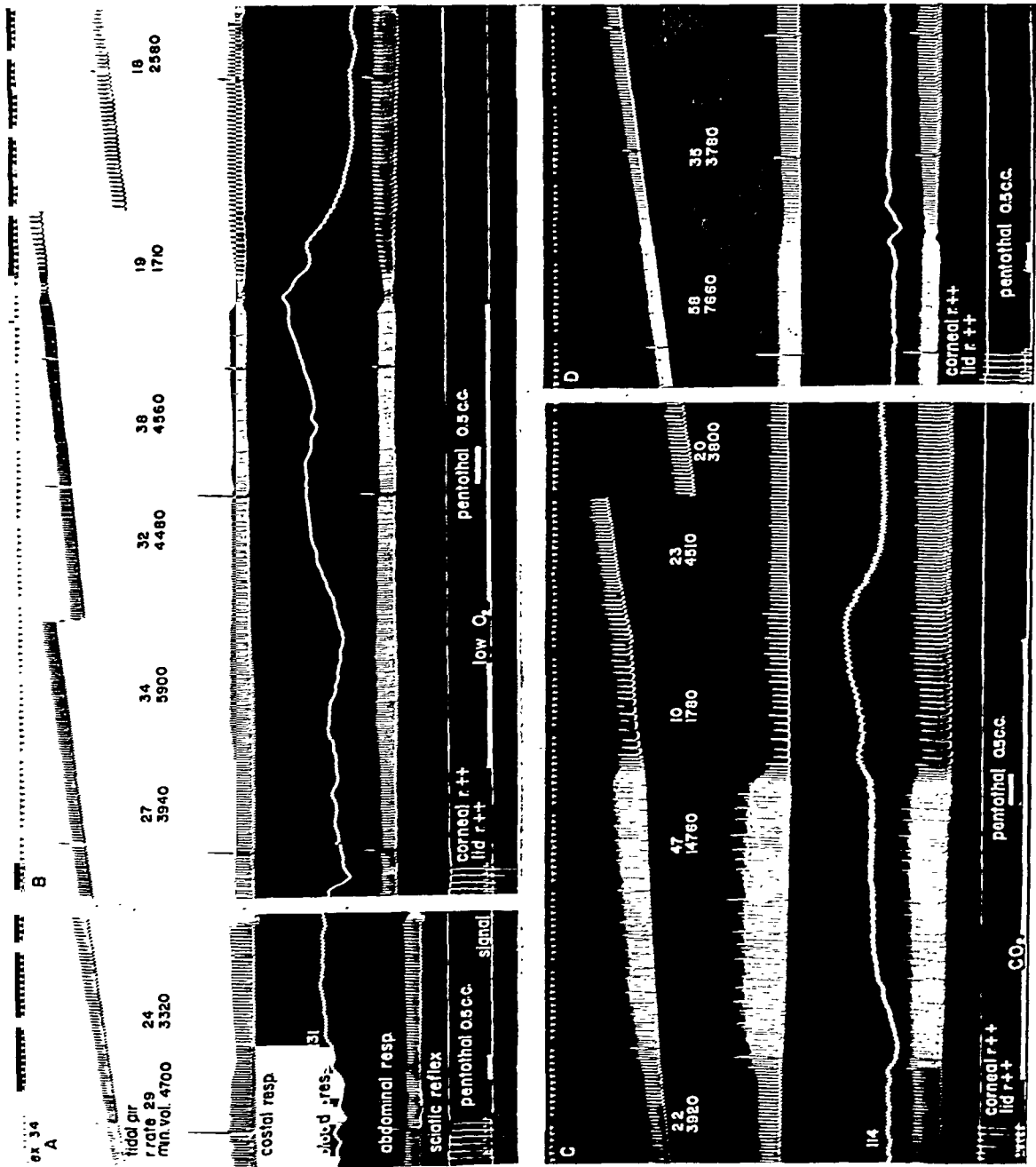


FIG. 3. PENTOTHAL (5 PER CENT SOLUTION) ANESTHESIA

Details are as for Figure 1. Weight of dog, 8.5 kgm. "Low oxygen" refers to 8 per cent



produce a fatal result even in a lightly anesthetized subject.

It is apparent from the data already presented that low oxygen has a stimulating action on the respiration over a wide range of anesthesia depth. It is also evident that, even when the subject is in good condition and only lightly anesthetized, the combination of low oxygen and modest dosage of barbiturate can produce a particularly malignant form of respiratory failure, one that is irreversible even when artificial respiration is promptly instituted and oxygen administered. It is important to emphasize, therefore, that low oxygen can first mask barbiturate depression and later add to the total depression. The masking effect is brought out in Figure 1, at the ends of Sections B, D, F, where sharp falls in ventilation are shown when the low oxygen is discontinued and room air is resumed. Figure 3 also illustrates the masking effect. Here, the dog weighed 8.5 kgm. Five per cent pentothal was administered intravenously. In Section A, 0.5 cc. (total dose, at that injection) in the lightly anesthetized animal altered the minute volume of respiration from 4700 cc. to 3320 cc. on room air. The rate was slowed from 29 to 24 per minute. A little later, when the animal had returned to about the same light level of anesthesia, it was shifted from room air to 8 per cent oxygen in the inspired air. The initial ventilation was 3940 cc., the rate 27. After four minutes on low oxygen this had changed to 4480 cc., with a rate of 32. At this point the same dose of pentothal used initially, 0.5 cc., was injected. Although the rate increased appreciably, from 32 to 38, the minute ventilation now was unchanged at 4560 cc. per minute; this contrasts sharply with the initial result. Clinically, one could not have detected any depressant effect of this second dose of pentothal. The respiration, unchanged in minute volume, increased a little in rate but would not have suggested a depressant effect of the pentothal; the blood pressure was not significantly altered by the agent. Yet it is clear from the earlier Section A and the later Section D that this dose of pentothal would, with a normal concentration of oxygen in the inspired air, have produced respiratory depression. In section B this effect was masked by the low oxygen. This masking effect becomes evident only when the hypoxia is relieved by substituting room air for the low oxygen. The truly depres-

sant effect then becomes obvious. In the example at hand the minute volume of 4560 cc. fell at once to 1710 cc.; the blood pressure declined rapidly, indicating that it had been maintained at its level by anoxia. The great sensitivity of these responses is indicated by the considerable effects produced under circumstances of normal oxygen intake by a very small dose of barbiturate on the one hand, and by the great depression of respiratory exchange effected by the shift from 8 per cent oxygen to room air on the other hand.

(3) The depressant effect of low oxygen was mentioned in the preceding section, and data were presented to indicate how serious the masking of barbiturate depression by low oxygen might be. It will now be shown that high oxygen during barbiturate anesthesia can also have serious consequences. The fact that a high arterial oxygen tension could effect a depressant action under barbiturates and certain other agents has long been known. Mosso (7), Henderson (5), Marshall and Rosenfeld (6), and others have observed it. Figure 4 demonstrates that this effect can be lethal when evipal is the anesthetic agent.

TABLE II  
*Weight of dog 11.3 kgm.*

Time	Inspired	Color of mucous membranes	Arterial oxygen content	Arterial CO <sub>2</sub> content	Rate	Minute ventilation
			<i>volume per cent</i>	<i>volume per cent</i>		
1:17 p.m.	Room air	Good	15.5	49.8	37	4880
1:22 p.m.						
1:23 p.m.	100% O <sub>2</sub>				28	3220
1:25 p.m.					31	3600
1:29 p.m.			17.3	51.2		
2:27 p.m.	Room air	Very cyanotic	7.0	69.5	12	1290
2:28 p.m.	100% O <sub>2</sub>					
2:29 p.m.		Cyanosis much less			3	320
2:30 p.m.	Infrequent gasps		4.8	78.3	0	
2:38 p.m.	Death					

In Figure 4, A, the immediate effect of 100 per cent oxygen, even though the color of the mucous membranes was good, was to lower the rate from 37 to 28 and the ventilation from 4880 cc. to 3220 cc., with small blood changes in oxygen and carbon dioxide content. Later, when the animal was deeply anesthetized (sciatic stimulation produced a visible but not recorded response), the blood

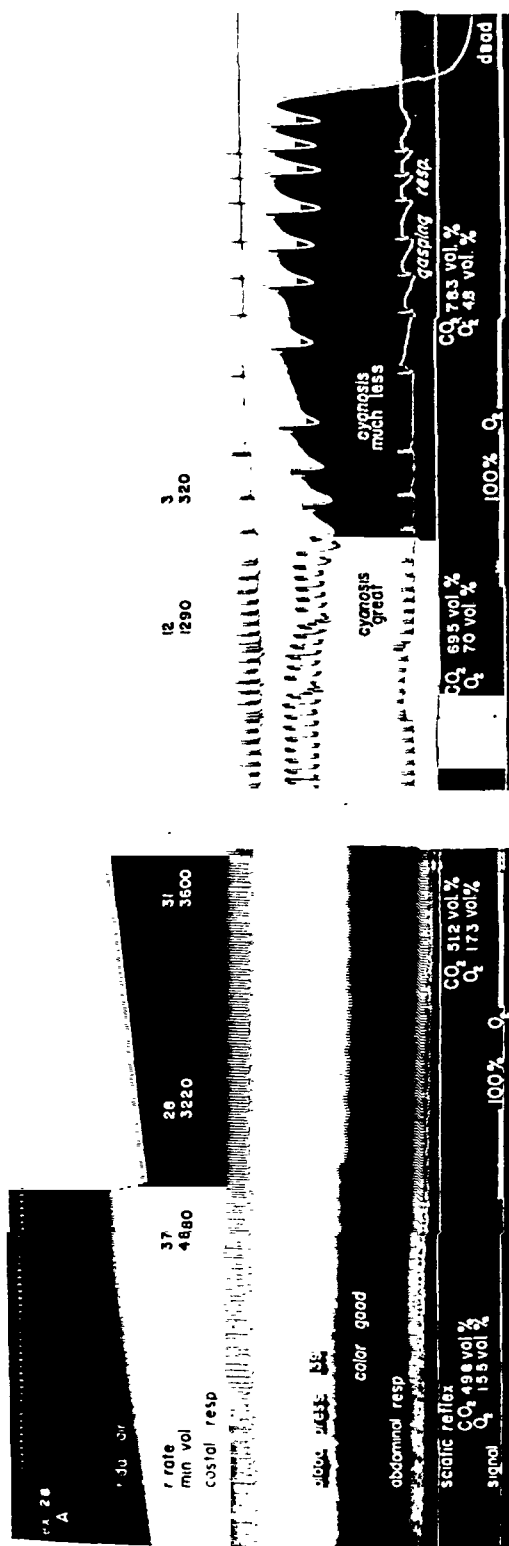


FIG. 4. EVISAL (10 PER CENT SOLUTION) ANESTHESIA  
Details are as for Figure 1. Weight of dog, 11.3 kgm.

oxygen seriously low, and the carbon dioxide content abnormally high, 100 per cent oxygen produced a distinct improvement in the animal's color; yet the respiratory rate, concomitant with the obvious improvement in oxygenation of the blood, dropped at once from 12 to 3 and quickly failed completely, although the heart continued to beat for at least a minute and a half following the last gasp.

(4) Respiratory alteration and failure following administration of barbiturates when the blood carbon dioxide content is high is shown in Figure 1, A, C,<sup>3</sup> E. These three sections represent light, moderately deep and deep anesthesia. (Note the sciatic reflex responses.) The dog used in this experiment weighed 9.8 kgm. Pentothal in 5 per cent solution was the anesthetic agent.

TABLE III

Figure	Time	Depth of anesthesia	Pulmonary minute volume of ventilation		
			Initial	After 4 minutes on 12% CO <sub>2</sub>	Immediately after additional pentothal
1, A	11:13 a.m.	Light	5,640	26,900	2,240 [1.0 cc.]
1, C	12:10 p.m.	Moderately deep	3,490	8,850	0 [0.8 cc.]
1, E	1:32 p.m.	Deep	2,030	4,160	0 [0.4 cc.]
1, D'	12:36 p.m.	Moderately deep			[0.8 cc.]

In passing, it will be observed that Figure 1, A, C, E and Table III demonstrate the gradual decrease of sensitivity of the respiratory center to a given carbon dioxide concentration in the inspired air. In Figure 1, A, four minutes of breathing 12 per cent CO<sub>2</sub> resulted in nearly a five-fold increase in ventilation; in Figure 1, C the effect was half (two and one-half-fold) what it had been in Figure 1, A; in Figure 1, E, four minutes of breathing 12 per cent CO<sub>2</sub> merely resulted in doubling the ventilation. This characteristic of the barbiturates has been discussed elsewhere by Moyer and Beecher. The point of immediate importance is that the respiratory center is less sensitive than normal to its natural stimulus, carbon dioxide. The carbon dioxide finally becomes, in

<sup>3</sup> In Figure 1, C, an example of sudden alteration of cardiac activity, probably due to heart block, can be seen. This was frequently encountered under pentothal under the circumstances of these experiments. It is being investigated further.

fact, a depressant. Therefore, it might be expected that additional doses of the barbiturate would have a tendency to produce respiratory failure when the carbon dioxide level of the blood was high, when otherwise this would not be so. Figures 1 and 1', D' show this to be the case. Respiratory failure is not produced in Figure 1, A by 1.0 cc. 5 per cent pentothal. At a deeper level of anesthesia a smaller dose (0.8 cc.) did cause the respiration to fail. Chronologically, the doses were given as follows: 9:00 a.m., 5.0 cc. of 5 per cent pentothal; 9:15, 1.0 cc.; 9:30, 1.0 cc.; 10:10, 1.0 cc.; 10:27, 1.0 cc.; 11:18, 1.0 cc.; 11:45, 1.0 cc.; 12:00, 1.0 cc.; 12:15, 0.8 cc.; 12:37, 0.8 cc.

It might be argued that the respiratory failure in Figure 1, C was due to the cumulative effects of the repeated doses. That this is not so is indicated by the fact that the respiration did not fail when the same size dose was administered later while the animal was at least as deeply anesthetized as in Figure 1, C, but was not on a high carbon dioxide intake, Figure 1', D' (preceding D' two doses of agent in addition to those listed had been administered at fairly close intervals).

Figure 8 illustrates in an animal breathing room air the enormous increase in ventilation effected by carbon dioxide stimulation in a subject lightly anesthetized with evipal (similar to Figure 1, A). Later, at a deeper level of anesthesia, the swift production of respiratory failure by a brief exposure to the same concentration of carbon dioxide is shown. Anoxia caused the blood pressure to rise. A moderate degree of negative pressure in the airway reestablished respiration. After a minute on negative pressure, enough carbon dioxide had been washed out and the carbon dioxide depression overcome to such an extent that the respiration could continue spontaneously when the negative pressure was relieved (10).

In Figure 9, A, the animal was deeply anesthetized and breathing 100 per cent oxygen. The initial respiratory rate was 6 with a minute ventilation of 1460 cc. The sciatic reflex response is interesting, in that it was more greatly depressed than could be accomplished with evipal alone without respiratory failure. It would appear safe to conclude that the animal was already suffering from carbon dioxide depression. If this were true, administration of synthetic carbon dioxide should result in further depression of respiration

and the sciatic reflex, not stimulation. This was the case. After three minutes of breathing 12 per cent carbon dioxide, the respiratory rate had slowed to 2 per minute, and the minute volume was reduced to 570 cc. At the time the carbon dioxide administration was discontinued, the rate of respiration was slowing rapidly, and almost at once failed completely. Following the respiratory failure, electrical stimulation of the sciatic nerve reestablished respiration. With the first stimuli no muscle response was recorded. The respiration resulting from the sciatic stimulation undoubtedly washed out a considerable part of the carbon dioxide which had been depressing the subject. The reappearance and the subsequent small but definite increase in the record of the reflex response support this.

The animal was still deeply anesthetized, however, and the respirations were only 4 per minute with a minute ventilation of 1060 cc. when the vagi were blocked with cold. While the rate remained the same, the amplitude of respiratory excursion increased to result in a minute volume of 1480 cc. Carbon dioxide was administered again, and again respiration failed, this time with the vagi blocked.

In Section B of Figure 9, recorded an hour and a half following Section A, the animal had become considerably lighter. (Observe the sciatic and the eye reflexes.) The carbon dioxide stimulus now increased the ventilation from 2140 cc. per minute to 3040 cc., rather than depressed it as it had in Section A. This was also true when the vagi were blocked. In A, with anesthesia deep, carbon dioxide depressed respiration; in B, with anesthesia lighter, respiration was stimulated. These changes occurred notwithstanding vagal blocks. These results were confirmed in other cases. It must be concluded from Figures 1, A, C, E, 8, and 9 that a high concentration of carbon dioxide in the inspired air enhances the depressant action of a given dose of barbiturate; this effect is increasingly great with deepening anesthesia.

(5) The early effect of low oxygen in masking the barbiturate depression, and the acutely fatal effects of severe anoxia during barbiturate anesthesia, have been demonstrated. The increased depression caused by given small doses of barbiturates when the blood carbon dioxide level is high has also been shown. It is reasonable to suppose

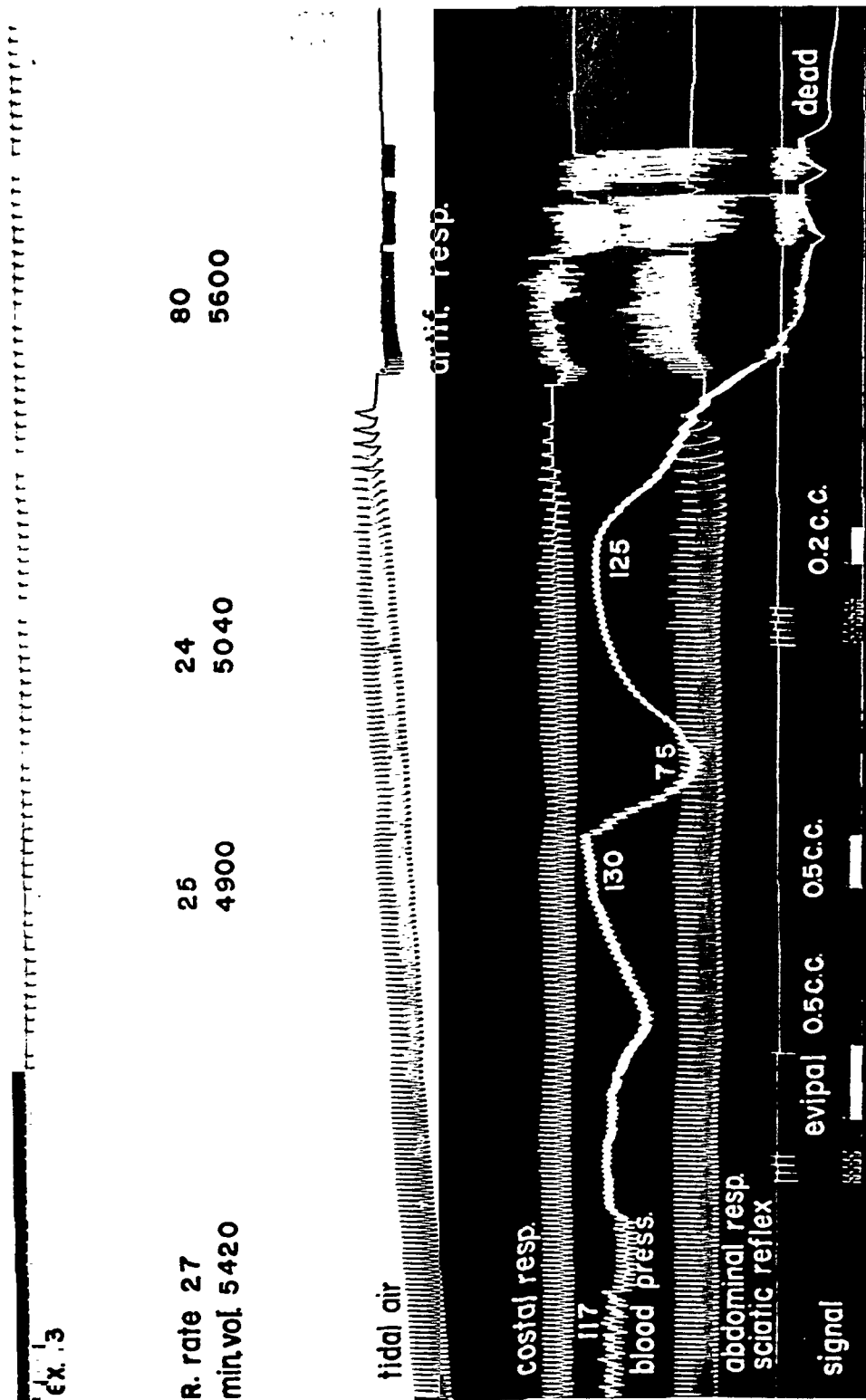


FIG. 5. EVIPAL (10 PER CENT SOLUTION) ANESTHESIA  
Details are as for Figure 1. Weight of dog, 10.0 kgm.

that the combined effects of low oxygen and high carbon dioxide in the arterial blood might be peculiarly serious. Such is the case. Figure 5 demonstrates a result of this combination. During the course of this experiment on a healthy, approximately one-year-old, 10 kgm. dog, it was observed that the Tissot valve in the expiratory half circuit had become stuck in the open position, with the effect that the dead space was greatly increased and no proper circulation of air took place through the soda lime. The animal was thus subjected to gradually increasing oxygen shortage and a gradual piling up of carbon dioxide. These effects were not clinically evident. An active sciatic reflex response was present and indicated that the animal was only moderately deeply anesthetized. The pulmonary ventilation was good with a respiratory rate of 27 and a minute volume of 5420 cc. The mean blood pressure was normal at 117 mm. Hg. Since the animal was not as deeply anesthetized as desired, it was decided to give more of the 10 per cent evipal solution; 0.5 cc. (total dose, *not* per kgm.) was administered. The changes produced in the respiration were so slight and so fleeting that they probably would not have been detected clinically. One minute following the administration of the evipal the respiratory rate was 25, the minute volume 4900 cc., and the blood pressure 130 mm. Hg, certainly reassuring. Since the animal was still quite obviously not yet deeply anesthetized, 0.5 cc. of the anesthetic solution was administered as shown, and again only a fleeting depression of the respiration and blood pressure was apparent. From the records obtained of these effects it is evident that the blood pressure suffered more severely than in the first instance. Yet it remained at a fairly low level for only about 20 seconds. It is hardly likely that this brief dip would have been detected clinically. If it had been, by the time the anesthetist checked it recovery would have taken place, and the first low reading would have been put down as error. The animal was still not deeply anesthetized, as shown by the record of the sciatic reflex just before the third dose was administered. At this time the respiratory rate was 24, the minute volume of respiration 5040 cc. and the blood pressure 125 mm. Hg. Certainly nothing was present that could have been detected clinically and that might have warned of impending disaster. Since the

animal was not yet as deeply anesthetized as desired, it was decided to administer a further small dose of evipal, 0.2 cc. The response to this was swift and completely unexpected. The respiration slowed at once and entirely failed within a minute. The blood pressure fell abruptly. Artificial respiration was promptly started and ventilation at the rate of 5600 cc. per minute was carried out. The heart continued to beat for two minutes following the respiratory failure and then stopped despite the vigorous artificial respiration. The mechanism of the death here and its clinical significance will be considered in the discussion.

Figure 4, B, also presents an example of death under conditions of high blood carbon dioxide and low blood oxygen. This will be discussed in conjunction with the example shown in Figure 5, already described.

(6) Figure 6 shows, during the breathing of 100 per cent oxygen, the increasing effect of a moderate elevation of pressure in the airway (5.0 cm.  $H_2O$ ) with deepening anesthesia, indicated by the diminution in the sciatic reflex response. In D, the increase in pressure by 5.0 cm.  $H_2O$  produced respiratory failure. The stimulating effect of negative pressure (5.0 cm.  $H_2O$ ) is also shown. In E, the respiratory depression was so great that respiration failed even under atmospheric pressure; sciatic stimulation produced two breaths. Negative pressure caused the respiratory activity to be resumed. Figure 7 demonstrates that the apnea produced by positive pressure is occasioned by a reflex mediated through the vagus; vagal cold blocks permitted the respiration to be resumed. Figure 8, B, illustrates the power of negative pressure to reestablish respiration which has failed as a result of carbon dioxide depression.

#### DISCUSSION

The factors responsible for the variability of response to a given dose of barbiturate, from one patient to another or in the same patient on different occasions, have in the main been obscure. It will be apparent from the data presented and the discussion to follow, that variations in oxygen supply and carbon dioxide content of tissues and blood can exert a powerful influence on the responses resulting from given doses of barbiturates. However, before these matters are dealt with, it should be observed in passing that a reciprocal



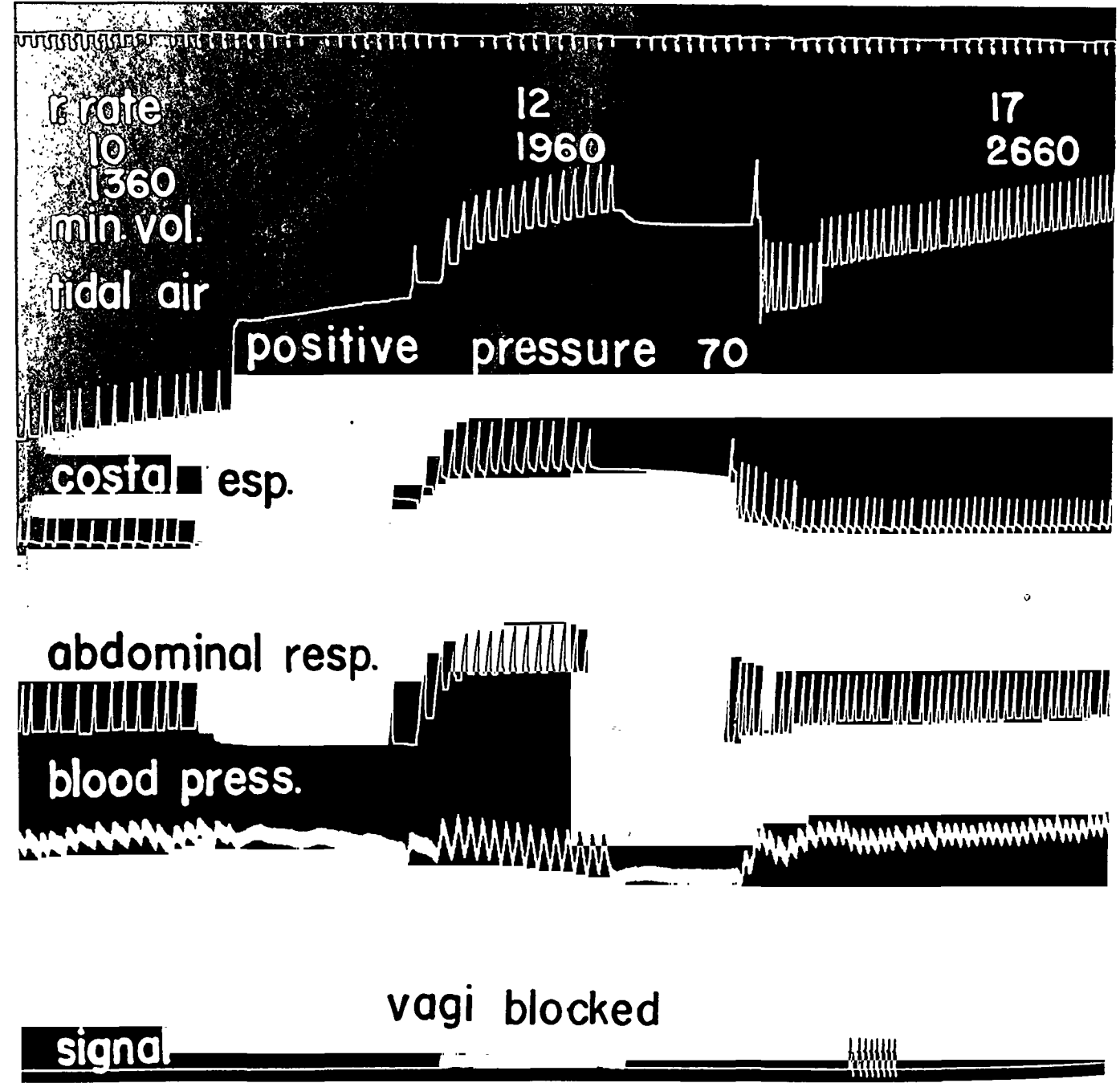


FIG. 7. EVIPAL (10 PER CENT SOLUTION) ANESTHESIA  
Details are as for Figure 1, except on 100 per cent oxygen. Weight of dog, 11 kgm.

relationship between dosage and pulmonary ventilation is apparent. It is plain that increasing dosage will lead to rather early respiratory failure. This holds both for evipal and pentothal. The tendency is demonstrated for the latter in Figures 1, B, D, F, where in round numbers the initial ventilation under light anesthesia was 6 liters per minute, under moderately deep anesthesia 4.5, and under deep anesthesia 3. In these 3 cases the effective respiratory impulses from the center have been reduced from 38 to 27 to 17 per minute.

The center loses much of its sensitivity to its major normal stimulus, carbon dioxide. This is demonstrated in Figure 1, A, C, E. In round numbers this is reflected in a decrease in total response above "normal" to the "standard" carbon dioxide stimulus, from 21 liters per minute to 5 to 2. Figure 8, A, B, illustrates the change from a tremendous stimulation to a frank depression with respiratory failure in response to a given concentration of carbon dioxide. Evipal was the anesthetic in this case.

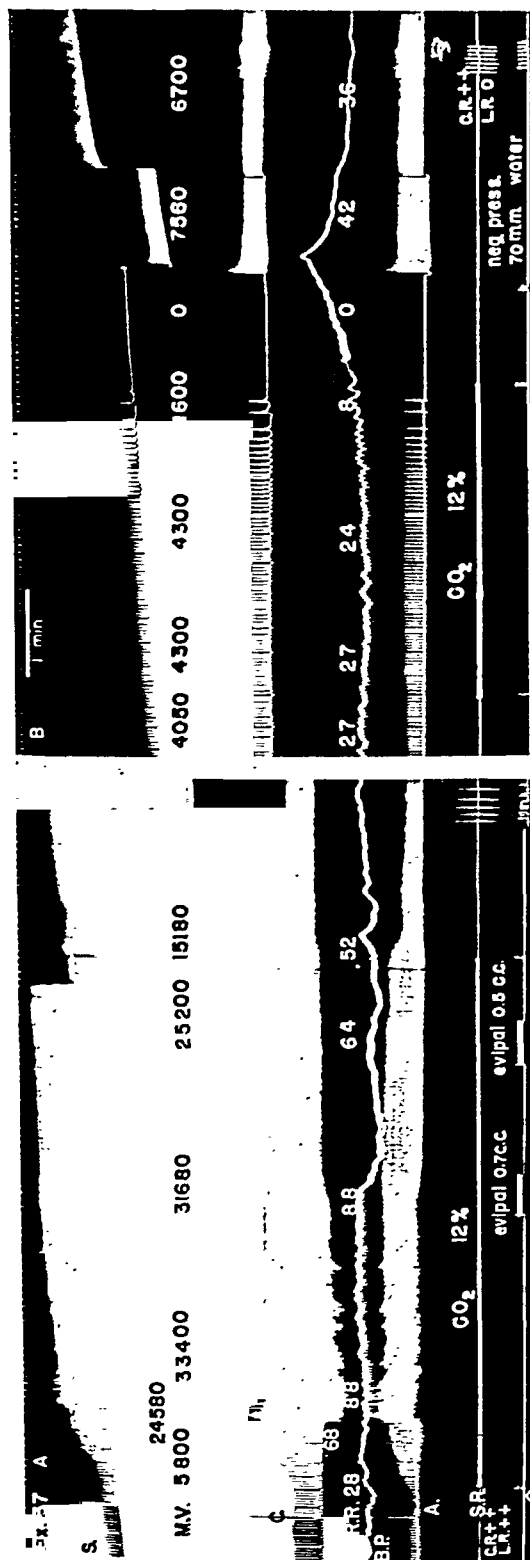


FIG. 8. EVIPAL (10 PER CENT SOLUTION) ANESTHESIA  
Details are as for Figure 1. Weight of dog, 10.5 kgm.

While the barbiturates rapidly diminish and finally eliminate the sensitivity of the respiratory center to carbon dioxide, the response to low oxygen is reduced very little as shown in Figure 1 and Table I. The same final minute volume response to low oxygen at the three widely differing levels of anesthesia suggests that, under moderate to deep barbiturate anesthesia, the respiration is chiefly maintained by mechanisms which are activated by low oxygen stimulation, which is in agreement with Schmidt (11), Cordier and Heymans (2), and Marshall and Rosenfeld (6). As demonstrated by Moyer and Beecher (8), central stimulation of low oxygen is important, although only under very light anesthesia, as well as peripheral stimulation, arising chiefly in the carotid and aortic bodies as shown by Heymans and others. It is interesting to observe that even at the deep level of anesthesia no loss of sensitivity to the low oxygen concentration employed is apparent, although the initial ventilations were in each case progressively smaller, presumably due to central depression by the barbiturate, resulting in the loss of sensitivity to the normal central stimulus, carbon dioxide.

The increasing effectiveness of small doses of the barbiturates is illustrated in Figure 1 and Table I. It will be recalled in the case of the volatile anesthetic agents, ether for example, that the concentration of this agent in the blood required to produce loss of consciousness is only one-third of that necessary to produce light surgical anesthesia, yet a small increase in ether concentration in the blood after the surgical level has been reached will carry the subject from light to dangerously deep anesthesia. In the case of the volatile agents, this clinical effect depends upon the approach of saturation of the lipid structures with the fat-soluble anesthetic agents. Whether a comparable "saturation" occurs in the case of the barbiturates is not known. If such were the case, it would be of great interest to know where the barbiturates are concentrated.

Inasmuch as the short-lasting barbiturates, evipal and pentothal, depress the central drive mechanism more than the reflex drives of respiration, the masking of serious barbiturate depression by oxygen tensions that are below normal is not surprising in view of the fact that low oxygen stimulates breathing reflexly (excepting, of course, those



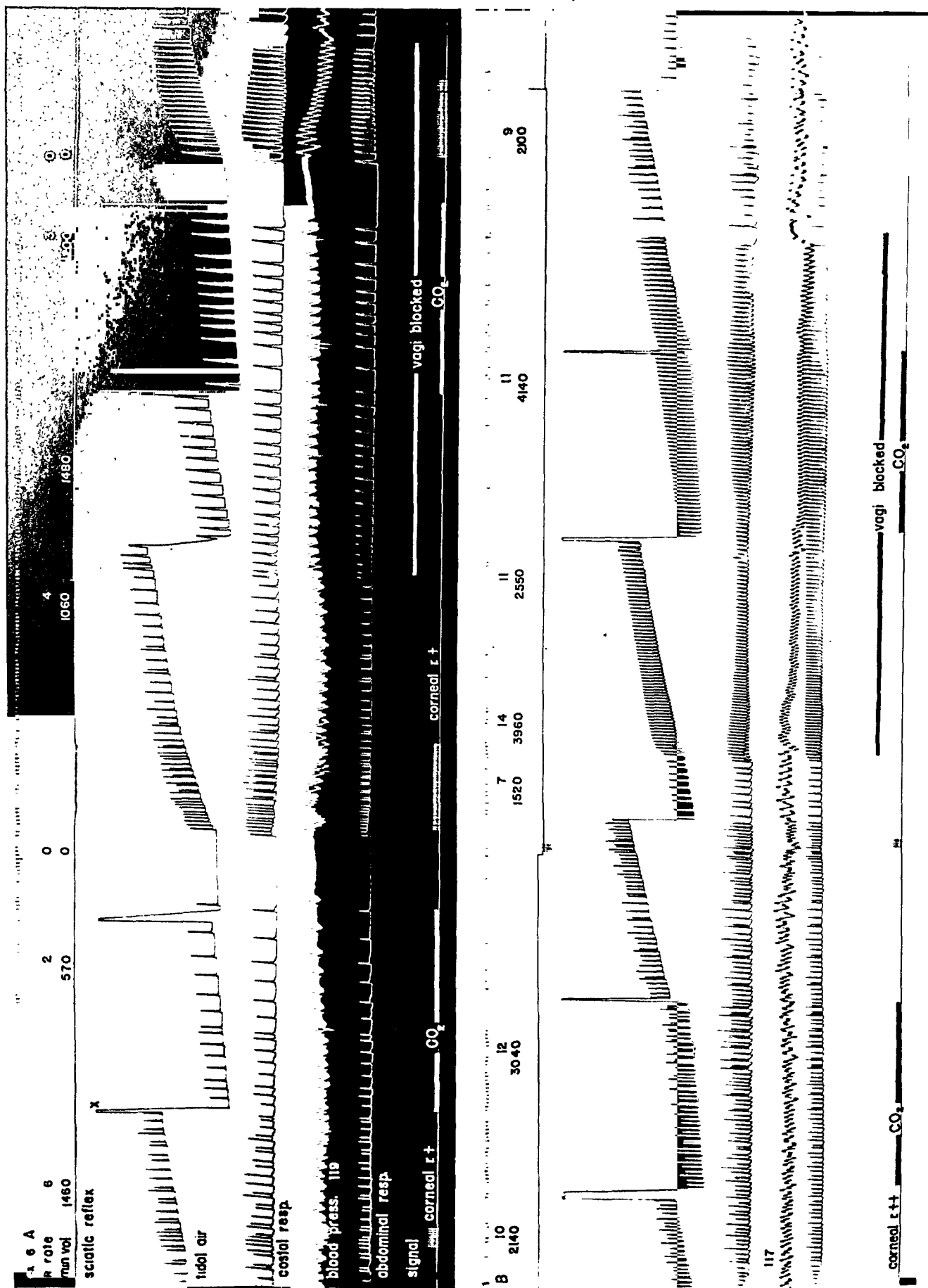


FIG. 9. EVISPAL (10 PER CENT SOLUTION) ANESTHESIA

Details are as for Figure 1, but animal breathing 100 per cent oxygen, except as indicated. Weight of dog, 12.2 kgm. Large excursions as at X indicate change of gas mixture by a method used in only a few experiments

cases where the central sensitivity to  $\text{cH}^+$  is very high and carotid and aortic sensitivity is very low). The clinical implications of this masking are so obvious that they need no discussion. A sudden increase in the oxygen content of the inspired air, even though it be slight, reveals, by virtue of the associated reduction of chemoreflex stimulation, the depressant action of the barbiturate (Figures 1, B, D, F and 3, B). Such respiratory depression, coupled with the already demonstrated loss of sensitivity of the respiratory center to carbon dioxide, can lead to a particularly serious form of depression. The sequence of events is probably as follows: Barbiturate anesthesia is administered to the subject. The sensitivity of the center to  $\text{H}^+$  is reduced. After the center's  $\text{H}^+$  sensitivity is greatly impaired or entirely destroyed the anoxic stimuli maintain pulmonary ventilation that is dependent upon oxygen level and the effectiveness of reflex drive. The anesthetist observes that the reflex drive is inadequate to maintain proper oxygenation if the subject's color is poor and he increases the oxygen concentration in the inspired air. The carotid and aortic body stimuli set up by the low oxygen are thus decreased, as the blood oxygen content approaches normal. Loss of the low oxygen stimuli allows the true state of affairs to become apparent to the discerning observer. The subject's respirations are found to be seriously depressed as a result of the barbiturate action; however, the high concentration of oxygen in the inspired air still maintains an adequate blood color and the vasomotor status is good. During this period the subject's good color keeps attention from the fact that the respiration is not adequate as far as the excretion of carbon dioxide is concerned, for the center has lost much of its ability to respond to the high concentration of carbon dioxide in the blood. The carbon dioxide piles up until it, too, becomes a true depressant (Figure 8, B). Suddenly the respiration fails. The high carbon dioxide blood content, having first stimulated, then depresses. Blood pressure finally falls precipitously. Irreversible changes swiftly take place and, notwithstanding artificial respiration, death occurs.

The effect of low oxygen in masking the true depressant action of the barbiturate must not be considered as in itself a good thing, for the dan-

gers inherent in a low oxygen intake are still present. They are too well known to need discussion. The masking effect will, of course, pass when the availability of oxygen in the tissues falls so low that the anoxia itself effects a depression. During the respiratory depression carbon dioxide piles up in the blood and tissues. In such a case, when the masked depression is carried to the breaking point, the additive effects of the depression caused by low oxygen, the depression caused by the high carbon dioxide, and the depression of the barbiturate, working together not only upon the respiratory center but upon the vasomotor center as well, swiftly produce changes which lead to death. Figure 4 illustrates the rapid failure of respiration while the blood color improved, with death due finally to a combination of low oxygen, high carbon dioxide and drug depression.

The circumstances which lead to the death of the animal pictured in Figure 5 might easily have their counterpart in the clinic (and probably often have had). Following the sudden death of a patient under barbiturate anesthesia, the statement is often heard that death was wholly unexpected and occurred without warning. The writers must admit to a considerable scepticism in the past as to the quality of the observation which allowed such statements to be made. It would seem that such an attitude is unjust, for in the laboratory at least it has repeatedly been possible to produce "sudden death without clinical warning". It is evident from Figure 5 that until the very end nothing was apparent in the condition of the only moderately deeply anesthetized subject that could have reasonably been detected clinically and have warned of impending death. This is to be explained, we believe, upon the basis just described of low oxygen masking the true depression of the barbiturate while carbon dioxide gradually accumulated to a depressant degree. The low oxygen, high carbon dioxide and the final, although very small, dose of barbiturate produced a depression of the respiratory and vasomotor centers which could not be overcome by vigorous artificial respiration.

The respiratory depression and failure produced by relatively slight elevation of pressure in the airway under the barbiturates during the breathing of 100 per cent oxygen, as shown in Figures 6 and 7, need further study before their

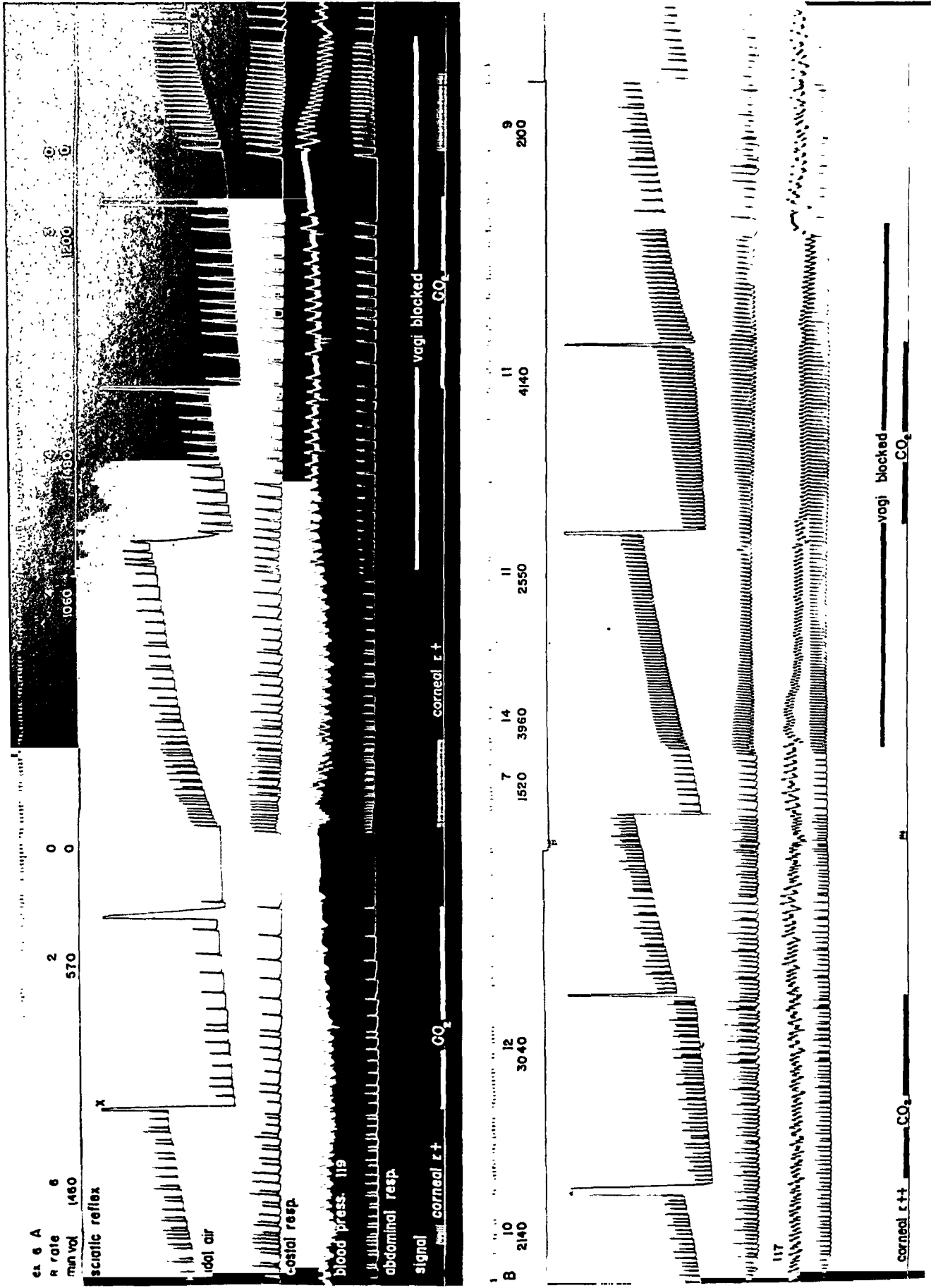


FIG. 9. EVIPAL (10 PER CENT SOLUTION) ANESTHESIA

Details are as for Figure 1, but animal breathing 100 per cent oxygen, except as indicated. Weight of dog, 12.2 kgm. Large excursions as at X indicate change of gas mixture by a method used in only a few experiments

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The respiratory depression and failure produced by relatively slight elevation of pressure in the airway under the barbiturates during the breathing of 100 per cent oxygen, as shown in Figures 6 and 7, need further study before their

full clinical significance can be estimated. For example, how applicable these findings are to momentary blockage of the airway by the tongue and to the probable alterations of vagal proprioceptive reflex balance of asthmatics is not clear. If this type of reflex respiratory failure is produced in asthmatics during barbiturate anesthesia, a contraindication to the use of these barbiturate agents would be evident on this basis alone, and a more complete explanation would be at hand for the vague clinical impression that barbiturate anesthesia should not be administered to asthmatics.

However, there is no need to speculate on this unexplored aspect of the asthmatic problem, for enough objective data are at hand to indicate how hazardous the use of these agents in asthmatics can be. The dangers of oxygen want and a high blood carbon dioxide level have been shown. These are the very conditions encountered in asthma. The alveoli are ventilated poorly and some of them not at all. The normal gaseous exchange is grossly interfered with. The alveolar air contains a high percentage of carbon dioxide and a low percentage of oxygen, and the blood contains a low oxygen content with retention of carbon dioxide. The tendency of carbon dioxide to pile up in the blood of the asthmatic is accelerated by the impairment of the sensitivity of the respiratory center to carbon dioxide by the barbiturate. As the barbiturates are administered, the peripheral chemoreceptors activated by the low oxygen stimuli mask the overdosage present. The high carbon dioxide blood content causes the level of serious overdosage to be reached sooner than would normally be the case. Suddenly the low oxygen stimuli are no longer adequate to maintain respiration; the low oxygen becomes of itself depressant. The low oxygen depression, the carbon dioxide depression and the barbiturate depression, acting together, swiftly damage the organism. It is no wonder that asthmatic patients "do not do well" under barbiturate anesthesia.

It is evident that there is no need to add a possible vagal proprioceptive reflex tending to produce respiratory failure to the already sufficient reasons why barbiturate anesthesia in asthmatics is dangerous. Certainly the above demonstration of respiratory failure as a result of a little increase in pressure in the airway offers further cogent reason why barbiturate anesthesia

should not be employed in patients who may require positive pressure anesthesia as in some types of thoracic surgery.

It follows from the remarks concerning asthma that the use of barbiturate anesthesia in emphysematous patients is ill-advised. While the tidal excursion in emphysema may be essentially normal, the residual air (as in the asthmatic) is greatly increased with corresponding decrease therein of oxygen tension and elevation of carbon dioxide to 50 to 60 mm. Hg, 7 to 8 per cent. The arterial blood saturation may be only a little below normal or it may go as low as 85 per cent (1). Why the gaseous exchange is impaired as much as it is in emphysema has not been completely explained. The *relative* insensitivity to carbon dioxide in emphysema further increases the hazard of barbiturate use. Morphine must be used with caution since it reduces the carbon dioxide sensitivity of the center.

Similar contraindications to the use of barbiturate anesthesia are apparent in the respiratory and blood gas exchange alterations effected by cardiac disease.

In diabetes mellitus accumulation of abnormal acids is found in the blood; on being buffered by plasma bicarbonate these acids disturb the  $\text{H}_2\text{CO}_3/\text{BHCO}_3$  ratio in the direction of acidemia. In uncompensated acidemia the hazards of the use of these agents are greater than in normal individuals.

Since one of the most important functions of the kidneys is the regulation of the acid-base balance of the plasma, acidemia frequently results from renal insufficiency. Therefore, the barbiturates are contraindicated for the reasons mentioned above. Factors such as these probably account for the fairly widespread reluctance to employ these barbiturates in patients with kidney disease, rather than any considerations of kidney excretion of the agents or their breakdown products, since kidney excretion does not appear to be of importance in the case of the short-lasting barbiturates.

Many other clinical conditions could be considered wherein the use of the barbiturates appears to be unwise. Such a list would include, surgical shock, among others, all conditions that result in a low blood or tissue oxygen or a high carbon dioxide blood level, or both.

## SUMMARY

The mechanisms of respiratory failure under evipal and pentothal anesthesia have been studied in experiments upon 43 dogs in order to get a better understanding of the factors involved in the variability of effect produced by given doses of the barbiturates. Three simultaneous records of respiration were made: tidal, costal and diaphragmatic, as well as a record of blood pressure. Depth of anesthesia was followed by records of the activity of a spinal reflex with frequent notes as to the state of the corneal and lid reflexes. Doubtless many still unknown factors are involved in the variability of action of these agents; however, several conditions frequently encountered clinically, which are capable of enhancing the toxic effects of the agents, are described.

Over a wide range of anesthesia, low oxygen in the inspired air effects a great increase in pulmonary ventilation. Although with increasing depth of anesthesia the initial minute volumes were progressively smaller, low oxygen produced the same final ventilation over the wide range of anesthesia studied (Figure 1, B, D, F). Even at the deep level of anesthesia considered, no loss of sensitivity to this stimulus was apparent with the oxygen concentration studied. Several demonstrations of the effect of a low oxygen tension in the blood in temporarily masking serious barbiturate depression are provided (Figures 1, B, D, F and 3, B). This effect is revealed by substituting room air for the low oxygen mixture being inspired. When the oxygen tension is very low it finally exerts a depressant effect which, on being added to the depressant action of the barbiturates, leads to death.

The effect of high oxygen in depressing the respiration which has been maintained in whole or in part by carotid and aortic mechanisms (activated by low oxygen) has been recognized, as far as the effect itself goes, for many years. This effect is present under evipal and pentothal. The elimination of the carotid and aortic activity by adequate oxygen tensions can be fatal (Figure 4).

Respiratory failure following the administration of relatively small doses of barbiturates when the blood carbon dioxide content is high is shown (Figure 1, C, E).

The sensitivity of the respiratory center to its normal stimulus, carbon dioxide, diminishes (Figure 1, A, C, E and Table III) and is finally lost under the barbiturates. The carbon dioxide finally becomes a serious depressant (Figure 8). A high concentration of carbon dioxide in the inspired air increases the sensitivity of the respiration to a given dose of barbiturate (Figure 1, A, C, E).

A combination of low oxygen and high carbon dioxide in the inspired air is particularly hazardous. Low oxygen masks the true depressant action of the barbiturate until later a point is reached when the low oxygen stimulation is no longer capable of supporting the respiration, but becomes itself depressant, and adds to the depressant action of the high carbon dioxide and the barbiturate, with a fatal outcome. This probably explains the "deaths without warning" described clinically (Figure 5).

The powerful influence exerted by various oxygen and carbon dioxide contents of the blood upon the response to a given dose of barbiturate is apparent. It is probable that variations in these gas tensions account in part for the well-known puzzling clinical variability of action of the barbiturates.

Respiratory depression and failure, as a result of a small elevation of pressure in the airway under barbiturate anesthesia and 100 per cent oxygen administration, are shown (Figure 6); they are due to a reflex mediated through the vagi (Figure 7). The clinical implications of this effect are discussed.

The increasing effectiveness of small doses of barbiturates, with increase in depth of anesthesia, and the reciprocal relationship of pulmonary ventilation to increasing barbiturate dosage are discussed.

The use of barbiturates appears to be contraindicated in conditions where the blood oxygen may be low or the carbon dioxide high. If these conditions occur during barbiturate anesthesia, the results can be disastrous. Not only is it important to maintain a good blood color under the barbiturates, it is equally important to see to it that the pulmonary ventilation is adequate in order to prevent the piling up of carbon dioxide in the tissues. The use of synthetic carbon dioxide is contraindicated as a respiratory stimulant during barbiturate anesthesia unless artificial respiration is being

given. In this case probably 5 per cent carbon dioxide should be administered in order to avoid excessive depletion of carbon dioxide.

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# CLINICAL STUDIES WITH THE AID OF RADIOACTIVE PHOSPHORUS. I. THE ABSORPTION AND DISTRIBUTION OF RADIO-PHOSPHORUS IN THE BLOOD AND ITS EXCRETION BY NORMAL INDIVIDUALS AND PATIENTS WITH LEUKEMIA<sup>1</sup>

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The purpose of this paper is (a) to indicate the amount of radio-phosphorus ( $P^{32}$ ) retained by various fractions of the blood of 4 normal individuals, 12 patients with myeloid, and 15 with lymphoid leukemia, and the variations in retention following the administration of radio-phosphorus when given orally and/or intravenously and when accompanied by varying amounts of non-radioactive phosphorus ( $P^{31}$ ); (b) to indicate the distribution of  $P^{32}$  in the bone marrow and in various fractions of white blood cells; and (c) to indicate the amount of radio-phosphorus excreted in urine and feces in these cases.

## MATERIALS AND METHODS

The radioactive phosphorus was produced by the Berkeley cyclotron (1). The 4 normal individuals were robust, ambulatory workmen with recently healed fractures, all of whom had received the same type and quantity of food over a period of from 1 to 8 weeks, and each of whom had a single regular bowel movement daily during the same period previous to the administration of  $P^{32}$ . It was impossible to control the diets or the time of excretion of the patients, all of whom were ambulatory. The blood withdrawn from veins was heparinized, cooled and centrifuged exactly 20 minutes at 1450 times gravity to insure constant volume. The buffy coat was aspirated, suspended in equal amounts of heparinized Ringer's solution and centrifuged exactly 20 minutes at 1450 times gravity. The plasma was then removed from the original tube and finally the red blood cells were extracted. Bone marrow obtained by sternal aspiration was heparinized and centrifuged, as described previously. In some instances, the nuclei were separated from the cytoplasm of the peripheral white blood cells by violent agitation for 20 minutes in cold 5 per cent citric acid (2). The phospholipids were extracted from the white blood cells by the use of ether, alcohol and reflux condensers, and the acid-soluble substances by use of ice-cold 5 per cent trichloroacetic acid, thereby leaving

the nucleoprotein-like substances as residue (3). The assays of radioactivity were made by use of an electrometer.

## RESULTS

The average percentages of the dose of administered  $P^{32}$  retained per 100 cc. of red blood cells, white blood cells and plasma of the normal individuals and of the patients are listed in Table I and illustrated in Figure 1.<sup>3</sup> The amounts and the method of administration of  $P^{32}$ , the number of cases studied, and the intervals in time are also noted. No attempts were made to correct for variations in the metabolic rate, blood volume, kidney and hepatic functions, diet, age, weight, etc. of the cases studied. In computing the averages (Table I), the determinations obtained from both acute and chronic cases of leukemia were used. The acute cases are indicated.

(a) *Retention of  $P^{32}$  in whole blood.* It can be observed that more  $P^{32}$  was retained, during

<sup>3</sup> All determinations in figures and tables were corrected for decay of radio-phosphorus (the half-life of which is 14.3 days) to date of administration. 1 microcurie ( $\mu$ c) or 1/1000 millicurie (Mc) is equal to 37,000 beta particles per second.

It must also be pointed out that all the doses of  $P^{32}$  administered to the patients discussed in this paper were "therapeutic" and not "tracer" in nature, for even the smallest dose (540 microcuries) administered caused a decrease in the white blood cell levels of the patient. Therefore, the values obtained in the various metabolic studies must be considered as having been "conditioned" by the biological effects of the beta radiation emitted by the  $P^{32}$  and the information about cellular metabolism, which was obtained incidental to therapeutic attempts, must be interpreted with reservation and caution.

Although the average doses of radio-phosphorus administered orally to the various groups (normal, myeloid and lymphoid) are not similar, those administered intravenously are comparable (Table I).

<sup>1</sup> These investigations have been aided by a grant from the John and Mary R. Markle Foundation.

<sup>2</sup> Wm. R. Kenan, Jr., Fellow.



TABLE I  
Average per cent of dose of P<sub>32</sub> administered orally and intravenously\* which was retained per 100 cc.  
of various fractions of blood of normal individuals and patients with leukemia

Oral administration											Intravenous administration										
2 Hours	4 Hours	6 Hours	24 Hours	48 Hours	72 Hours	96 Hours	5 Days	8 Days	10 Days	Num- ber of cases stud- ied*	Num- ber of cases stud- ied	2 Hours	4 Hours	6 Hours	24 Hours	48 Hours	72 Hours	96 Hours	5 Days	8 Days	9 Days
0.093	0.131 0.103	0.1026	0.0736 0.108 0.099	0.0626 0.100 0.0465	0.0419 0.084 0.0608	0.02	0.037	0.045		2 9 10	Whole blood Normal Myeloid Lymphoid	2	0.170	0.1686	0.1726	0.08		0.0308			
												10	0.177	0.13	0.131	0.129		0.103	0.082	0.0263	0.089
0.0981 0.029 0.15	0.098 0.121	0.1313 0.0899	0.1286 0.151 0.1098	0.1039 0.1068 0.0607	0.0426 0.0853 0.069	0.030	0.056	0.0112		2 9 10	Red blood cells Normal Myeloid Lymphoid	2	0.2303	0.2336	0.1993	0.132		0.0693			
												10	0.195	0.23	0.218	0.135		0.103	0.087	0.0393	0.072
0.021 0.118	0.117	0.0343 0.088	0.0554 0.210	0.1396 0.258	0.1410 0.248	0.1433 0.216	0.162	0.083		2 9 10	White blood cells Normal Myeloid Lymphoid	2	0.396	0.342	0.308	0.224	0.169	0.1223	0.144	0.0932	
												10	0.332	0.396	0.308	0.224	0.169	0.1223	0.144	0.0932	
0.0716 0.080	0.021 0.069 0.054	0.021 0.027 0.018	0.0115 0.0212 0.0166	0.016 0.0279 0.014	0.015 0.016 0.010	0.008 0.003 0.012	0.018	0.012		2 9 10	Plasma Normal Myeloid Lymphoid	2	0.448	0.451	0.452	0.591	0.450	0.112	0.247	0.1800	
												10	0.276	0.451	0.452	0.591	0.450	0.385	0.247	0.267	0.64
0.0716 0.080	0.021 0.069 0.054	0.021 0.027 0.018	0.0115 0.0212 0.0166	0.016 0.0279 0.014	0.015 0.016 0.010	0.008 0.003 0.012	0.018	0.012		2 9 10	Plasma Normal Myeloid Lymphoid	2	0.075	0.0219	0.018	0.020		0.0149	0.007	0.007	
												10	0.133	0.03	0.027	0.024	0.022	0.021	0.025	0.019	0.032
0.0716 0.080	0.021 0.069 0.054	0.021 0.027 0.018	0.0115 0.0212 0.0166	0.016 0.0279 0.014	0.015 0.016 0.010	0.008 0.003 0.012	0.018	0.012		2 9 10	Plasma Normal Myeloid Lymphoid	2	0.075	0.0219	0.018	0.020		0.0149	0.007	0.007	
												10	0.133	0.03	0.027	0.024	0.022	0.021	0.025	0.019	0.032

\* See table of Administrations.

## \* Administrations †

Oral				Intravenous		
Mgm. of $\text{Na}_2\text{PO}_4$	Microcuries of $\text{P}^{32}$	Cases		Cases	Microcuries of $\text{P}^{32}$	Mgm. of $\text{Na}_2\text{PO}_4$
600	Average	1	<i>Normal</i>	1	Average	600
600	1500	2		2	1500	600
	1500				1500	
50	850	1	<i>Myeloid</i>	1	765	100
830	1000	2		2	765	3000
150	2300	3		3	850	50
2000	2300	4		4	1140	150
1250	4700	5		5	1260	150
3000	4700	6		6	1950	300
2800	5960	7		7	2000	143
2400	7200	8		8	2000	2000
20	12600	9		9	2350	300
				10	2350	2000
	4623				1543	
5180	2440	1	<i>Lymphoid</i>	1	540	150
450	3000	2		2	1360	150
3000	3000	3		3	1900	150
3000	3050	4		4	1998	180
1500	3300	5		5	2160	180
750	4000	6		6	2250	300
142	4270	7		7	2550	2000
300	5000	8		8	2600	300
750	5000	9		9	2600	2000
3480	11680	10		10	5000	450
	4474				2295	

† The cases recorded here will be described individually and in detail elsewhere.

the period studied, in the whole blood of the patients than in that of the *normal individuals*, and that the concentration reached higher levels in both groups when the  $\text{P}^{32}$  was administered intravenously. This last feature was found to be true in all of the fractions of the blood.

(b) *Retention of  $\text{P}^{32}$  in red blood cells.* The same features were found as discussed under (a).

(c) *Retention of  $\text{P}^{32}$  in white blood cells.* The concentration of  $\text{P}^{32}$  in the white blood cells of the patients was considerably higher than that in the white blood cells of the normal individuals. The metabolic rates of the white blood cells were not determined. The concentration of  $\text{P}^{32}$  in the white blood cells of the leukemic patients (particularly the lymphoid cells) was higher following the administration of  $\text{P}^{32}$  by the intravenous than by the oral route.

(d) *Retention of  $\text{P}^{32}$  in plasma.* The concentration of  $\text{P}^{32}$  was higher in the plasma after in-

travenous than after oral administration for a short period of time only—approximately 2 to 4 hours. This finding was present in the normal individuals and in the patients.

Figure 2 also shows that there is more retention of radio-phosphorus in blood fractions following intravenous than following oral administration in individual patients receiving  $\text{P}^{32}$  by both routes, and that when relatively large amounts of  $\text{P}^{31}$  accompany the radioactive phosphorus, regardless of route of administration, less of the latter is retained by the fractions. One patient (Case 34) retained about as much  $\text{P}^{32}$  in the blood fractions after its oral administration, when accompanied with a small amount of  $\text{P}^{31}$ , as when the same quantity of radio-phosphorus was administered intravenously in the presence of more than 10 times more  $\text{P}^{31}$ .

Figure 3 shows that with one exception (R.B.C. of Case 23) the retention of  $\text{P}^{32}$  was less in the

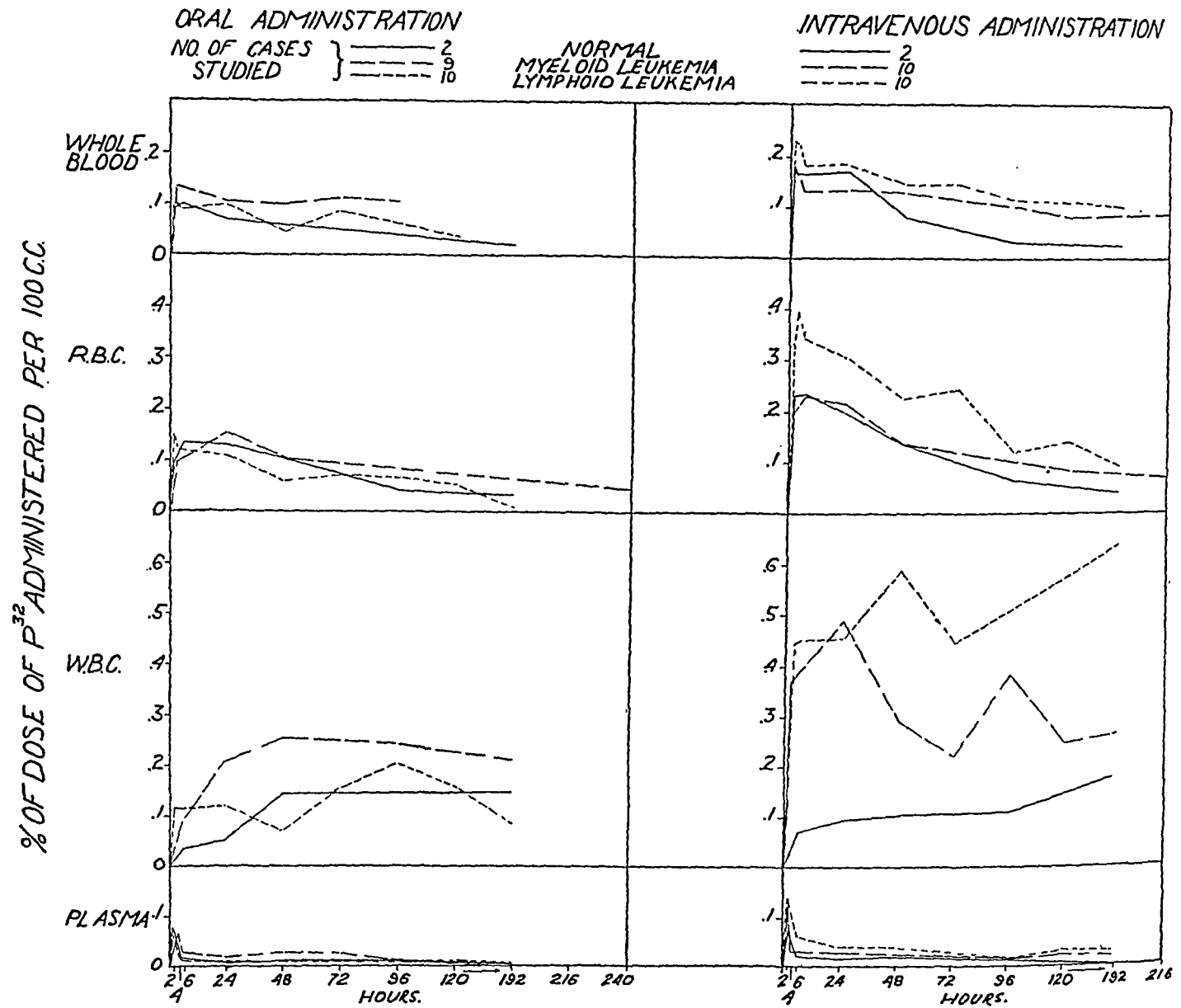


FIG. 1. AVERAGE RETENTION, IN BLOOD FRACTIONS, OF P<sup>32</sup> ADMINISTERED ORALLY AND INTRAVENOUSLY IN NORMAL INDIVIDUALS AND IN PATIENTS WITH LEUKEMIA

blood fractions when large amounts of P<sup>31</sup> accompanied the P<sup>32</sup> than when small amounts of non-radioactive phosphorus were introduced at the same time.

Table II indicates that P<sup>32</sup> is retained in the marrow in slightly higher concentrations than corresponding fractions in the peripheral blood at the same time period. The nucleated cells of the marrow include the nucleated red cells.

Table III indicates that the volumes of nuclei and cytoplasm of lymphocytes are about equal, while in myelocytes the nuclei comprise but about 1/5 to 1/3 of the volume of the cell. The ratio of the amounts of P<sup>32</sup> retained in the lymphocyte

nuclei to the cytoplasm is about 1 to 1, while in the myelocytes the ratio is 4 to 1 in the cases studied. Constant volumes were obtained by centrifuging samples for exactly 20 minutes at 1450 times gravity.

Table IV indicates that up to 48 hours after administration of P<sup>32</sup> the greatest concentration occurs in the acid-soluble fraction of leukemic white blood cells. In 2 patients (Cases 28 and 67), it was noted that the concentration of P<sup>32</sup> in the phospholipid and nucleoprotein fractions gradually increased following the administration of radio-phosphorus, while that in the acid-soluble fraction decreased. At the end of a period of 96

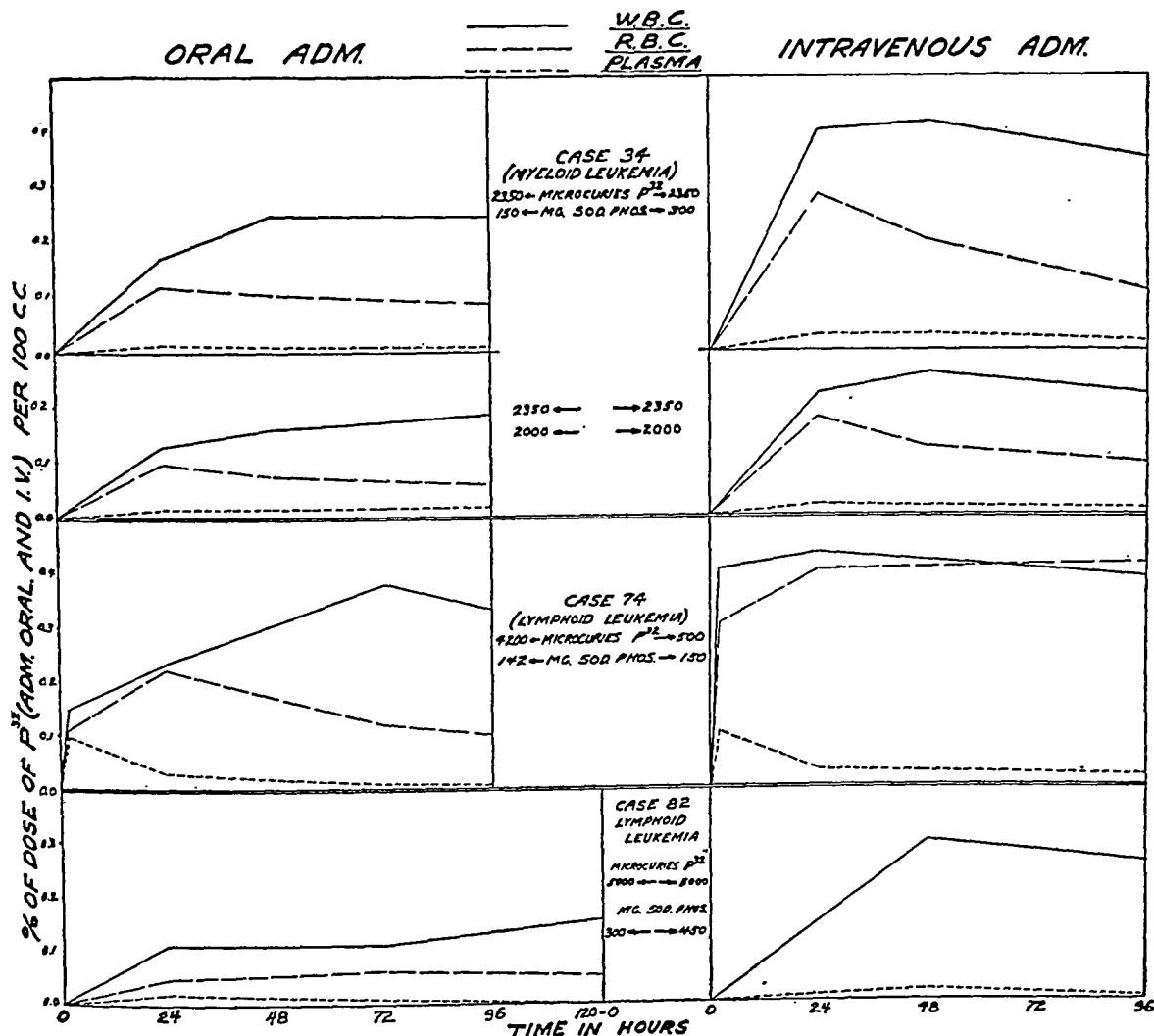


FIG. 2. VARIATIONS IN RETENTION, IN BLOOD FRACTIONS, OF  $P^{32}$  ADMINISTERED INTRAVENOUSLY AND ORALLY WHEN ACCOMPANIED BY LARGE AND SMALL AMOUNTS OF SODIUM PHOSPHATE

hours after the administration, the concentration of  $P^{32}$  in the nucleoprotein fraction was equal to or greater than the acid-soluble fraction in 4 of the 5 cases studied.

Figure 4 and Table V indicate the average per cent of the dose of administered  $P^{32}$  excreted in the urine and feces. When  $P^{32}$  is administered orally, from 15 to 50 per cent is excreted in the urine and feces in both normal individuals and patients during a 4- to 6-day period. In normal individuals the same percentages are excreted when  $P^{32}$  is administered intravenously, but in the patients from 5 to 25 per cent is excreted. When

administered orally, the greater part of the  $P^{32}$  is excreted in the feces; when intravenously, a very small but definite amount is excreted in the feces. Normal individuals excrete large quantities in the urine following intravenous administration. In leukemic patients radio-phosphorus is probably more quickly fixed in the pathological tissues and cells (3, 4).

#### DISCUSSION

It has been observed (3, 5) that leukemic mice and their tissues retain more radio-phosphorus than normal mice and their tissues, that the con-

TABLE IV

*Retention of radio-phosphorus in the phospholipid, acid-soluble and nucleoprotein fractions of leukemic cells*

Case number	Type of leukemia	Millicuries of $P^{32}$ administered	Route of administration	Hours after administration	Fractionation of white blood cells		
					Phospho-lipids	Acid-soluble	Nucleo-protein
67	Lymphoid	5	Oral	12	0.0048	$\mu\text{c. per cc.}$ 0.0465	0.0081
				24	0.0099	0.0435	0.0111
				48	0.0162	0.0417	0.0147
				96	0.0228	0.0187	0.0393
61	Lymphoid	2	Intravenous	96	0.0068	0.0079	0.0248
				4	0.0018	0.0195	0.0027
				24	0.0039	0.0190	0.0058
				48	0.0037	0.0160	0.0077
28	Myeloid	1.95	Intravenous	96	0.0057	0.0117	0.0105
				12	0.0055	0.0064	0.0036
				120	0.0018	0.0016	0.0018
				48	0.0031	0.0042	0.0065
14	Myeloid	0.87	Intravenous	120	0.0036	0.0035	0.0059
				120			
21	Myeloid	0.76	Intravenous	48			
				120			

TABLE V

*Average per cent of dose of  $P^{32}$  administered orally and intravenously (see below \*) which was excreted in the urine and feces of normal individuals and patients with leukemia*

Days	1	2	3	4	5	6	Number of cases studied*		Number of cases studied*	1	2	3	4	5	6
								<i>Urine</i>							
	8.66	1.83	1.50	0.93	1.07	0.83	2	Normal	2	27.16	4.26	1.99	1.58	1.50	1.11
	5.11	1.71	0.909	0.707	0.634	0.634	7	Myeloid	4	5.50	2.55	3.88	0.97	0.23	
	3.86	1.63	0.98	0.87			2	Lymphoid	6	4.19	1.81	0.86	0.96	0.56	0.4
								<i>Feces</i>							
	17.76	1.55	0.30	0.06		0.03		Normal		0.089	0.35	0.24	0.16	0.02	0.03
	11.05	5.79	4.64	1.746	1.08	1.09		Myeloid		0.25	0.16	0.27	0.14		0.03
	2.33	9.75	9.3	9.28				Lymphoid		0.022	0.177	0.638	0.484	0.35	0.68

\* Administrations

Oral					Intravenous			
Mgm. of $\text{Na}_2\text{PO}_4$	Microcuries of $P^{32}$		Cases		Cases	Microcuries of $P^{32}$		Mgm. of $\text{Na}_2\text{PO}_4$
		Average					Average	
600	1500		1	<i>Normal</i>	1	1500		600
600	1500	1500	2		2	1500	1500	600
930	3850		1	<i>Myeloid</i>	1	870		150
1000	4000		2		2	1140		150
3000	4700		3		3	1260		150
3000	4700		4		4	1950	1305	300
	4700		5					
2800	5960		6	<i>Lymphoid</i>				
20	12600	5787	7					
					1	540		150
750	4000		1		2	1360		150
142	4270	4135	2		3	1900		150
					4	1998		180
					5	2160		180
					6	5000	2159	450

centration of radio-phosphorus in the nucleoprotein fraction of leukemic cells of mice increased, while that of the acid-soluble fraction decreased during a 4-day period, that large quantities of non-radioactive phosphorus, when accompanying radio-phosphorus on administration, tend to reduce the amount of  $P^{32}$  retained in the bodies of mice. Similar findings are apparent in humans, as reported in this paper.

The following features were also noted: (a) more radio-phosphorus is retained by patients with leukemia when it is administered intravenously than when given orally; (b) marrow retains radio-phosphorus in higher concentrations than blood per unit volume and at the same time period; and (c) relatively greater concentrations of  $P^{32}$  occur in the nuclei than in the cytoplasm of myeloid leukemic cells, while no differences in retention of  $P^{32}$  were noted in the nuclei and cytoplasm of the lymphoid cells studied. From the findings presented above phosphorus apparently passes from the acid-soluble substances of leukemic white blood cells (presumably through enzymatic carrier systems) to substances of nucleoprotein and phospholipid character. The practical point to be emphasized is, that if high concentrations of radio-phosphorus are to be obtained in circulating white blood cells,  $P^{32}$  should be introduced intravenously and it should be accompanied by the smallest

amount of non-radioactive phosphorus possible. Reducing the phosphorus intake in the diet should also be considered.

#### SUMMARY

1. The variations in the retention of administered radio-phosphorus in blood of normal individuals and leukemic patients due to routes of administration and to amounts of accompanying non-radioactive phosphorus are presented.

2. The amounts of radio-phosphorus excreted in the urine and feces, after its administration both orally and intravenously, are given.

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STUDIES ON NEOPLASMS WITH THE AID OF RADIOACTIVE PHOSPHORUS.<sup>1</sup>  
III. THE PHOSPHORUS METABOLISM OF THE PHOSPHOLIPID, ACID  
SOLUBLE AND NUCLEOPROTEIN FRACTIONS OF VARIOUS  
TISSUES OF NORMAL AND LEUKEMIC MICE FOLLOW-  
ING THE ADMINISTRATION OF "TRACER" AND  
"THERAPEUTIC" DOSES OF RADIO-  
PHOSPHORUS

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"Tracer" doses of radio-phosphorus ( $P^{32}$ ) are small amounts which are conceivably insufficient to cause significant changes in the metabolism of the animal's cells in which they are retained, while "therapeutic" doses are large amounts of  $P^{32}$  which significantly alter the metabolism of cells because of the quantity of beta-radiation spontaneously emitted. A previous paper (1) presented the retention of radio-phosphorus, which had been administered intraperitoneally, in "phospholipid", "acid soluble" and "nucleoprotein" fractions of various tissues of normal mice and mice with generalized lymphomata. This paper presents the retention of  $P^{32}$  in the same fractions of similar groups of mice following the administration of both "tracer" and "therapeutic" doses of radio-phosphorus.

#### MATERIALS AND METHODS

The same materials, methods and techniques were used in this experiment as in the experiment mentioned above. The radio-phosphorus was produced by the Berkeley cyclotron (2) and converted into a sodium phosphate (15 mgm. per cc.) solution which was sterilized and assayed for radioactivity. It has been determined (unpublished data) that a dose of radio-phosphorus emitting 70 microcuries of beta radiation given intraperitoneally (as a sodium phosphate solution containing  $P^{32}$ ) is lethal for mice weighing 20 grams in one to two weeks. The "tracer" dose of  $P^{32}$  used in this experiment consisted of  $\frac{1}{2}$  cc. of a sodium phosphate solution that contained 7.5 mgm. of  $Na_2HPO_4$  at a pH of 7.4, and emitted 8 microcuries on the day of administration. The "therapeutic" dose used in this experiment was of the same volume, the same concentration of  $Na_2HPO_4$ , and the same pH, but

emitted 80 microcuries of beta radiation on the same day. Five-tenths per cent, 1 per cent, 2 per cent, 5 per cent and 10 per cent quantities of both solutions, accurately measured, were kept as reference samples and their radioactivities were compared with those of the fractions studied, thus obviating calculations for decay of radio-phosphorus. All measurements of radioactivity were made by the use of a DuBridge type of ion chamber electrometer.

One hundred and seventy-six highly inbred Strong A strain mice (each weighing between 18 and 22 grams) were housed in thirty-two clean wire-bottomed cages, were fed almost identical amounts of Purina dog chow and oats ten days before and during the experiment, and were divided into 16 groups. Eight groups of 10 mice each (5 males and 5 females) served as the control or normal series. Eight groups of 12 mice each (6 males and 6 females), which served as the leukemic series, were injected intraperitoneally with 15 million cells of a lymphoma (3) fourteen days before being sacrificed. This lymphoma "takes" 100 per cent in the Strong A strain, produces uniform generalized leukemic infiltrations of the liver, spleen and lymph nodes and, in addition, grows in the peritoneal cavity as a very cellular, non-necrotic localized tumor mass. Six of the 176 died before completion of the experiment and were discarded. The remaining 170 were sacrificed twelve, twenty-four, thirty-six and forty-eight hours after intraperitoneal injections of "tracer" (8 microcuries per mouse) and "therapeutic" (80 microcuries per mouse) doses of radio-phosphorus.

The spleens, livers, lymph nodes (central and peripheral) both of the normal and the leukemic animals and the intraperitoneal tumor masses of the leukemic animals of the 16 groups respectively were pooled, weighed and prepared for analysis. The respective carcasses were pooled and ashed at 400 degrees Centigrade. The "phospholipid" fraction of the four tissues was extracted by means of ether, alcohol and reflux condensers, the "acid soluble" by cold 5 per cent trichloroacetic acid, and the residue was considered the "nucleoprotein" fraction (1).

The amounts of  $P^{32}$  excreted by these animals were unfortunately not determined.

<sup>1</sup> This investigation has been aided by a grant from the John and Mary R. Markle Foundation.

<sup>2</sup> William R. Kenan, Jr., Fellow.



TABLE I

*Retention of radio-phosphorus in "phospholipid", "acid soluble" and "nucleoprotein" fractions of various tissues and remainder of bodies of normal mice and mice with lymphoma twelve, twenty-four and forty-eight hours following its intraperitoneal administration in small and large doses*

			$\mu\text{c. of P}^{32}$ administered intraperitoneally				Hours after administration of $\text{P}^{32}$ (expressed in per cent of dose per gram wet weight)			
					12	24	36	48		
Spleen	Normal	Phospholipid	$\begin{Bmatrix} 8 \\ 80 \end{Bmatrix}$	$\begin{Bmatrix} 0.834 \\ 0.854 \end{Bmatrix}$		0.689	1.19	0.592 0.545		
		Acid soluble	$\begin{Bmatrix} 8 \\ 80 \end{Bmatrix}$	$\begin{Bmatrix} 2.0 \\ 1.64 \end{Bmatrix}$		$\begin{Bmatrix} 1.61 \\ 1.10 \end{Bmatrix}$	$\begin{Bmatrix} 1.27 \\ 1.01 \end{Bmatrix}$	0.888 0.872		
		Nucleoprotein	$\begin{Bmatrix} 8 \\ 80 \end{Bmatrix}$	$\begin{Bmatrix} 2.02 \\ 1.64 \end{Bmatrix}$		$\begin{Bmatrix} 2.32 \\ 1.64 \end{Bmatrix}$	2.08	1.76 1.25		
	Leukemic	Phospholipid	$\begin{Bmatrix} 8 \\ 80 \end{Bmatrix}$	$\begin{Bmatrix} 0.877 \\ 0.702 \end{Bmatrix}$		$\begin{Bmatrix} 0.898 \\ 0.791 \end{Bmatrix}$	$\begin{Bmatrix} 0.932 \\ 0.864 \end{Bmatrix}$	0.748		
		Acid soluble	$\begin{Bmatrix} 8 \\ 80 \end{Bmatrix}$	$\begin{Bmatrix} 2.49 \\ 2.13 \end{Bmatrix}$		$\begin{Bmatrix} 1.64 \\ 1.53 \end{Bmatrix}$	$\begin{Bmatrix} 1.64 \\ 1.49 \end{Bmatrix}$	1.32		
		Nucleoprotein	$\begin{Bmatrix} 8 \\ 80 \end{Bmatrix}$	$\begin{Bmatrix} 3.26 \\ 2.81 \end{Bmatrix}$		$\begin{Bmatrix} 3.43 \\ 2.97 \end{Bmatrix}$	$\begin{Bmatrix} 3.85 \\ 3.12 \end{Bmatrix}$	2.87		
Liver	Normal	Phospholipid	$\begin{Bmatrix} 8 \\ 80 \end{Bmatrix}$	$\begin{Bmatrix} 2.16 \\ 1.76 \end{Bmatrix}$		$\begin{Bmatrix} 1.63 \\ 1.32 \end{Bmatrix}$	$\begin{Bmatrix} 1.36 \\ 1.20 \end{Bmatrix}$	0.985 0.947		
		Acid soluble	$\begin{Bmatrix} 8 \\ 80 \end{Bmatrix}$	$\begin{Bmatrix} 1.47 \\ 0.920 \end{Bmatrix}$		$\begin{Bmatrix} 1.08 \\ 0.802 \end{Bmatrix}$	$\begin{Bmatrix} 1.02 \\ 0.745 \end{Bmatrix}$	0.672 0.532		
		Nucleoprotein	$\begin{Bmatrix} 8 \\ 80 \end{Bmatrix}$	$\begin{Bmatrix} 0.368 \\ 0.460 \end{Bmatrix}$		0.571	$\begin{Bmatrix} 0.683 \\ 0.558 \end{Bmatrix}$	0.640 0.550		
	Leukemic	Phospholipid	$\begin{Bmatrix} 8 \\ 80 \end{Bmatrix}$	$\begin{Bmatrix} 1.74 \\ 1.31 \end{Bmatrix}$		$\begin{Bmatrix} 1.44 \\ 1.30 \end{Bmatrix}$	1.28	1.17 1.01		
		Acid soluble	$\begin{Bmatrix} 8 \\ 80 \end{Bmatrix}$	$\begin{Bmatrix} 2.02 \\ 1.36 \end{Bmatrix}$		$\begin{Bmatrix} 1.37 \\ 0.954 \end{Bmatrix}$	$\begin{Bmatrix} 1.12 \\ 1.10 \end{Bmatrix}$	1.18 0.887		
		Nucleoprotein	$\begin{Bmatrix} 8 \\ 80 \end{Bmatrix}$	$\begin{Bmatrix} 1.27 \\ 1.27 \end{Bmatrix}$		$\begin{Bmatrix} 1.62 \\ 1.34 \end{Bmatrix}$	$\begin{Bmatrix} 1.99 \\ 1.37 \end{Bmatrix}$	1.87 1.62		
Lymph nodes	Normal	Phospholipid	$\begin{Bmatrix} 8 \\ 80 \end{Bmatrix}$	$\begin{Bmatrix} 0.431 \\ 0.292 \end{Bmatrix}$		$\begin{Bmatrix} 0.563 \\ 0.470 \end{Bmatrix}$	0.824	0.794 0.474		
		Acid soluble	$\begin{Bmatrix} 8 \\ 80 \end{Bmatrix}$	$\begin{Bmatrix} 1.54 \\ 1.12 \end{Bmatrix}$		$\begin{Bmatrix} 0.988 \\ 0.760 \end{Bmatrix}$	0.706	0.775		
		Nucleoprotein	$\begin{Bmatrix} 8 \\ 80 \end{Bmatrix}$	$\begin{Bmatrix} 1.14 \\ 0.820 \end{Bmatrix}$		$\begin{Bmatrix} 1.03 \\ 0.850 \end{Bmatrix}$	$\begin{Bmatrix} 1.16 \\ 0.858 \end{Bmatrix}$	1.13 0.882		
	Leukemic	Phospholipid	$\begin{Bmatrix} 8 \\ 80 \end{Bmatrix}$	$\begin{Bmatrix} 0.786 \\ 0.447 \end{Bmatrix}$		0.434	$\begin{Bmatrix} 0.777 \\ 0.592 \end{Bmatrix}$	0.776 0.621		
		Acid soluble	$\begin{Bmatrix} 8 \\ 80 \end{Bmatrix}$	$\begin{Bmatrix} 1.87 \\ 1.57 \end{Bmatrix}$		1.01	$\begin{Bmatrix} 1.62 \\ 0.635 \end{Bmatrix}$	0.632		
		Nucleoprotein	$\begin{Bmatrix} 8 \\ 80 \end{Bmatrix}$	$\begin{Bmatrix} 2.18 \\ 2.04 \end{Bmatrix}$		2.41	$\begin{Bmatrix} 2.44 \\ 2.42 \end{Bmatrix}$	2.52 2.42		

 $\mu\text{c.}$  = microcurie. $\text{P}^{32}$  = radio-phosphorus.

TABLE 1—Continued

		$\mu\text{c. of P}^{32}$ administered intraperitoneally	Hours after administration of $\text{P}^{32}$ (expressed in per cent of dose per gram wet weight)			
			12	24	36	48
Lymphoma	Phospholipid	{ 8	0.604	0.711	0.803	0.791
		{ 80	0.458	0.618	0.683	0.666
	Acid soluble	{ 8	2.59	1.24	1.54	1.35
		{ 80	1.63	1.36	1.21	0.845
	Nucleoprotein	{ 8		2.38	3.45	3.34
		{ 80	1.19	2.53	2.53	2.11
Carcass	Normal	{ 8	*	*	*	*
		{ 80	2.06 (9)	1.52 (10)	1.79 (9)	1.17 (10)
	Leukemic	{ 8	1.52 (10)	1.40 (10)	1.24 (10)	0.900 (10)
		{ 80	2.31 (10)	2.04 (12)	2.31 (12)	1.78 (12)
		{ 8	1.74 (12)	1.79 (11)	1.67 (11)	1.74 (12)
		{ 80				

\* Number of mice on which above data were obtained.

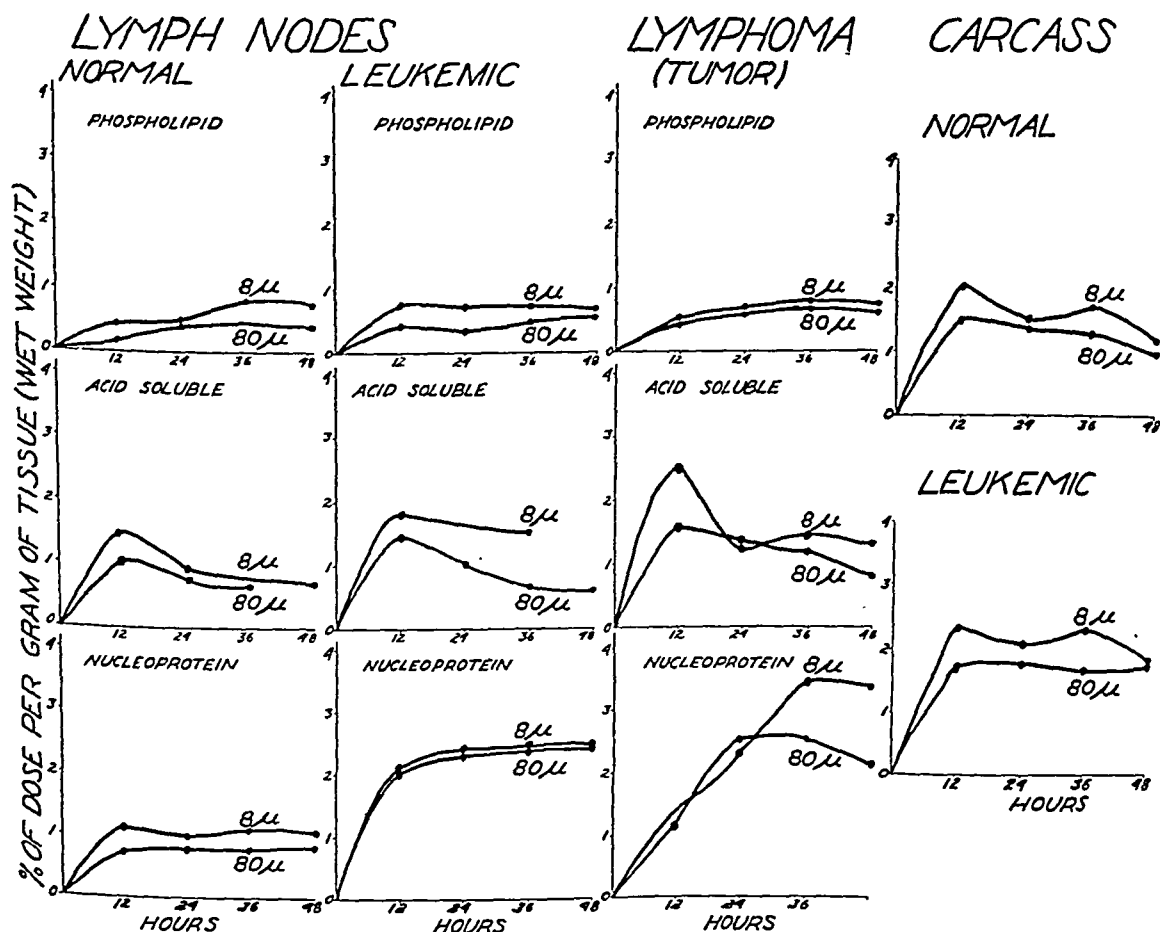


FIG. 1. THE COMPARATIVE RETENTION OF RADIO-PHOSPHORUS IN FRACTIONS OF TISSUES AND CARCASSES OF NORMAL AND LEUKEMIC ANIMALS 12, 24, 36 AND 48 HOURS FOLLOWING THE INTRAPERITONEAL ADMINISTRATION OF  $\text{P}^{32}$  EMITTING BOTH 8 AND 80 MICROCURIES OF BETA RADIATION PER MOUSE

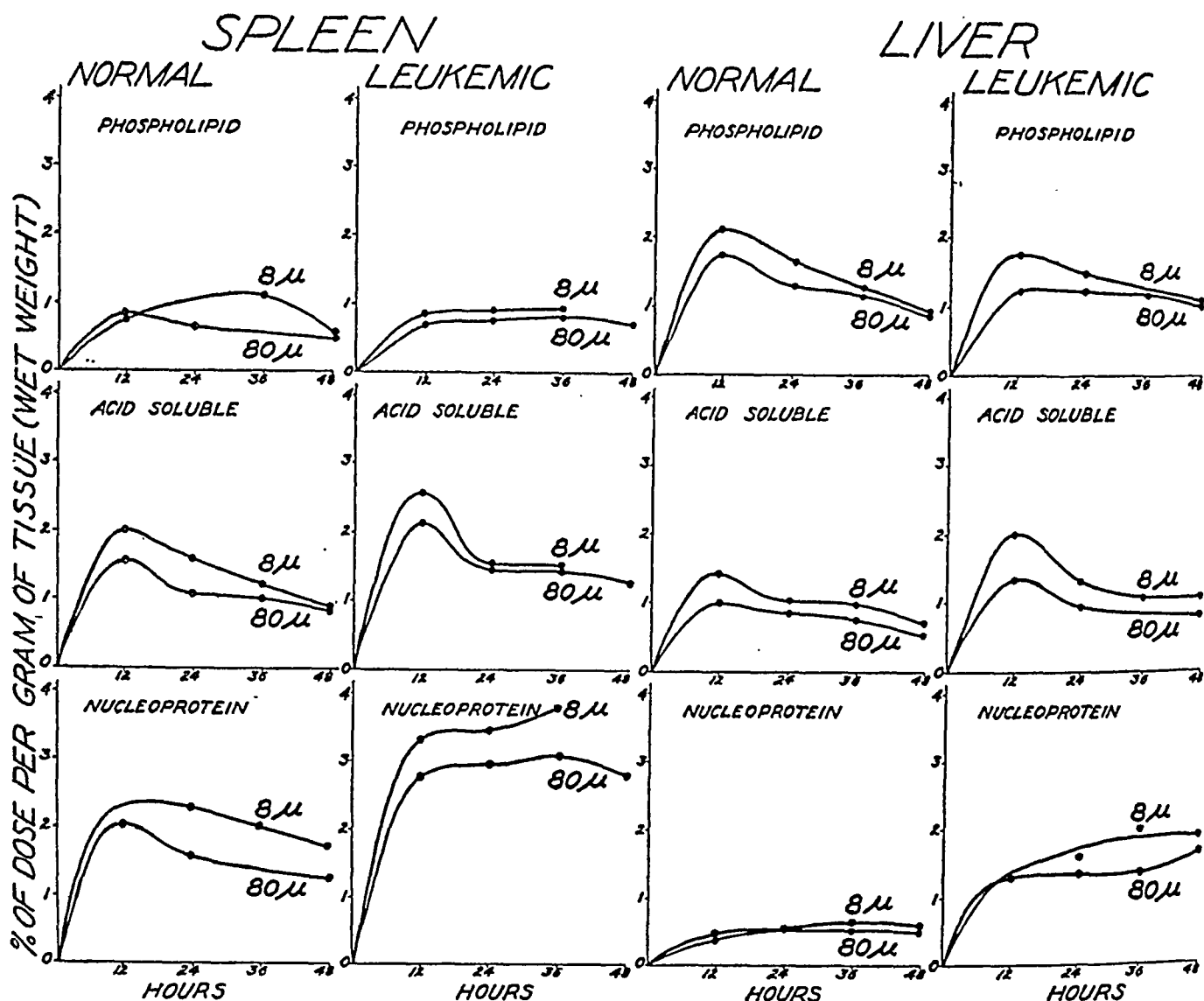


FIG. 2. THE COMPARATIVE RETENTION OF RADIO-PHOSPHORUS IN FRACTIONS OF TISSUES AND CARCASSES OF NORMAL AND LEUKEMIC ANIMALS 12, 24, 36 AND 48 HOURS FOLLOWING THE INTRAPERITONEAL ADMINISTRATION OF  $P^{32}$  EMITTING BOTH 8 AND 80 MICROCURIES OF BETA RADIATION PER MOUSE

### RESULTS

The results are listed in Table I and are illustrated in Figures 1 and 2.

The amounts (as per cent of the dose of  $P^{32}$  administered per gram of tissue-fresh wet weight) of radio-phosphorus retained in the various fractions of the four tissues and in the carcasses were consistently less following the "therapeutic" dose (80 microcuries per mouse) than those following the "tracer" dose (8 microcuries per mouse). In the majority of instances this difference was noted twelve hours after administration and persisted at the forty-eight-hour period also. The difference was perhaps slightly greater in the "nucleoprotein" fractions than in the "phospholipid" and "acid soluble" fractions. Although the leukemic

animals retained more  $P^{32}$  than the corresponding normal animals, the differences between levels of retention of  $P^{32}$  were quite similar in the corresponding fractions of the tissues of the 2 groups following "tracer" and "therapeutic" doses, respectively.

### DISCUSSION

These results indicate that, when large doses of radio-phosphorus are used, the effects of the irradiation can be measured. Therefore, in interpreting the results of metabolic investigations, it must be known whether large or small doses were used if radioactive agents were employed in obtaining these results. Since the results reported here, when 8 microcuries per animal were used,

are identical at the forty-eight-hour period with those when 5.5 microcuries were used (1) and almost identical with those when 30 microcuries and 50 microcuries per animal were used,<sup>3</sup> it would seem safe to conclude that 8 microcuries per animal is a safe "tracer" dose for mice on which such studies are to be made.

The results also indicate that there is no difference in radiosensitivity of the metabolic processes studied in the normal animals when compared with those of the leukemic animals.

This technique could well be used as a method to compare the radiosensitivity of various types of cellular metabolism both in normal and neoplastic tissues, and it may prove to be a valuable method of comparing the effects on these tissues of different types of radiation such as x-radiation or neutron radiation.

<sup>3</sup> See footnote, page 60, reference 1.

#### SUMMARY

Less  $P^{32}$  was retained in the "phospholipid", "acid soluble" and "nucleoprotein" fractions of spleen, liver and lymph nodes, and the carcasses both of normal mice and mice with lymphoma after intraperitoneal administration of large or "therapeutic" doses of radio-phosphorus than when small or "tracer" doses were given.

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# EXPERIMENTAL AND CLINICAL STUDIES ON GRAMICIDIN<sup>1</sup>

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A bactericidal substance isolated by Dubos (1) from a soil bacillus has a marked bactericidal action against gram-positive bacteria. This substance is toxic for laboratory animals when administered by the intravenous route (2). We have recently shown that one of the toxic effects of this substance is its hemolytic activity. The crude substance (tyrothricin) consists of two fractions, tyrocidine and gramicidin, as reported by Hotchkiss and Dubos (3). The observations of Dubos were based on experiments in which bacterial cultures, as well as studies on animals, were employed. The present study deals with (1) observations on the effect of the bactericidal substance and its fractions on the growth of a number of gram-positive organisms in tissue culture media, (2) observations on the hemolytic property of gramicidin, and (3) observations on the clinical use of gramicidin. The tissue culture method offers two chief advantages: first, one may observe the effect of the substance on pathogenic bacteria growing in the presence of tissue fragment, serum and tissue extract; second, one may observe the effect of the substance on the growth or maintenance of various types of cells and on erythrocytes suspended in the tissue culture clot. It seems likely that the conditions under which this study was made more closely approach those which obtain *in vivo* than do conditions usually employed in bacteriologic studies.

The tissue culture preparation used in the bactericidal studies was similar to the modified Maximow technic described by King, Henschel and Green (4). The culture was planted on a 22 mm. round coverslip and consisted of a drop of heparinized rabbit's plasma and three drops of tissue extract made by extracting seven-day chick embryos with rabbit's serum. To the preparation was added an explant from a mesenteric lymph node of a rabbit; each explant measured approximately 1.5 mm. across. The total volume of each culture was approximately 0.2 cc. Bacterial cultures used in this study were

grown in dextrose brain broth. One cubic centimeter of rabbit's serum was added to brain broth cultures of pneumococci and hemolytic streptococci. Dilutions of young dextrose brain broth cultures were made in plain broth and added to the tissue extract in the proportion of one part of a suspension of bacteria in plain broth to forty parts of tissue extract. The final concentration of the original bacterial culture in the tissue culture clot was one to ten million. This inoculum resulted in the appearance of twenty or more bacterial colonies in each tissue culture preparation with the exception of certain strains of hemolytic streptococci which grew only in the vicinity of the tissue fragment. It was necessary to use a dilution of one to one million cultures of these strains.

Three fractions of the substance elaborated by the soil bacillus were used: the crude bactericidal substance, tyrothricin, and its two fractions, gramicidin and tyrocidine. The lots of tyrothricin used in this study were furnished respectively by Dr. René J. Dubos and by Sharp and Dohme. They were found to be similar in bactericidal activity. Dr. Dubos kindly furnished us with purified gramicidin and purified tyrocidine. Also used in this study were samples of purified gramicidin and tyrocidine prepared from tyrothricin by Osterberg according to the method of Hotchkiss and Dubos. Since these products of the soil bacillus are insoluble in saline solutions, suitable suspensions of the material dissolved in 95 per cent alcohol were made in Tyrode's solution. Such suspensions containing varying amounts of bactericidal substance were added in the ratio of 1 to 10 to plasma and tissue extract used in preparing the tissue culture clot. The greatest amount of bactericidal substance used was 300 micrograms per cubic centimeter of medium. Similar dilutions of 95 per cent alcohol in Tyrode's solution were used in control cultures. Four cultures were prepared for each experimental condition. Cultures were incubated at 37° C. in a specially constructed circulation type incubator (5), and final readings were made after the culture had been incubated for forty-eight hours. The cultures were examined by using a magnification of seven diameters. The least amount of bactericidal substance which would completely prevent the appearance of bacterial colonies in all four cultures after incubation for forty-eight hours was determined for each bacterial strain tested.

Tissue cultures to which bactericidal substances and bacteria had been added, but which showed no evidence of bacterial growth in forty-eight hours, were put into tubes of dextrose brain broth to which 1 cc. of horse serum or rabbit's serum had been added. These cultures were incubated at 37° C. for five days in order to determine whether or not any viable bacteria were present.

<sup>1</sup> Read before the meeting of the American Society for Clinical Investigation, Atlantic City, New Jersey, May 5, 1941.

The following bacterial species have been studied: (1) four strains of smooth, encapsulated *Diplococcus pneumoniae*, that is, one of type I, two of type III, and one of type XIX; (2) five strains of group A Lancefield hemolytic streptococcus;<sup>2</sup> (3) two strains of the viridans group of streptococci (*Streptococcus salivarius*) isolated from the blood of patients with endocarditis; (4) four strains of *Streptococcus faecalis*; and (5) six strains of *Staphylococcus aureus*.

The results of bactericidal tests are shown in Table I. There was a considerable degree of spe-

TABLE I  
*Amounts of bactericidal substance causing inhibition*

Organism	Number	Tyro- thricin <i>μg. per cc.</i>	Grami- cidin <i>μg. per cc.</i>	Tyro- cidine <i>μg. per cc.</i>
<i>Diplococcus pneumoniae</i>				
Type I		2.5	1.0	100
Type III	1	1.0	0.5	20
Type III	2	1.0	1.0	20
Type XIX			2.5	40
Hemolytic streptococcus (Group A Lancefield)	1	10	5.0	80
	2		5.0	100
	3		10.0	80
	4		5.0	100
	5		20.0	120
<i>Streptococcus viridans</i> group	1		10	60
	2		60	120
<i>Streptococcus faecalis</i>	1	20	40	300
	2		60	*
	3		20	260
	4		60	300
<i>Staphylococcus aureus</i>	1		100	140
	2		—*	—*
	3		300	—*
	4		—*	—*
	5		—*	—*
	6		300	300

\* Not inhibited by 300  $\mu$ g. of bactericidal substance.

cies resemblance in the reaction of the various strains to gramicidin. Dubos also observed this in his *in vitro* experiments. The amount of gramicidin necessary to inhibit the growth of all of the representatives of each species was as follows: *Diplococcus pneumoniae*, 0.5 to 2.5 micrograms per cubic centimeter; hemolytic streptococcus, 5 to 20 micrograms per cubic centimeter; *Streptococcus faecalis*, 20 to 60 micrograms per cubic

centimeter; *Streptococcus salivarius*, 10 to 60 micrograms per cubic centimeter; and *Staphylococcus aureus*, 100 to 300 micrograms per cubic centimeter. Three strains of *Staphylococcus aureus* were not inhibited by 300 micrograms of gramicidin per cubic centimeter. For the most part, a slightly greater amount of tyrothricin had to be used to cause inhibition of bacterial growth as compared to gramicidin. In general, it was necessary to use a much greater concentration of tyrocidine than of gramicidin to obtain the same degree of inhibition (Figures 1 and 2).

Although no bacterial growth occurred in cultures containing a sufficient amount of bactericidal substance, not all of the bacteria were killed. When such cultures were placed in brain broth, serum added, and incubated at 37° C. for several days, an occasional tube would show growth of the organism originally introduced into the tissue culture. This occurred when any of the bactericidal fractions were used and for all species except the *Staphylococcus aureus*. In experiments with this species, however, there were few negative clots available for study. Little is known concerning the mode of action of gramicidin. In the tissue culture preparations the presence of 1 to 10 mgm. of para-aminobenzoic acid per cubic centimeter did not inhibit the action of gramicidin on pneumococci.

#### *Studies on hemolysis*

The hemolytic effect of gramicidin has recently been reported from our laboratories (6). This observation was made concerning hemolysis that occurred when the crude substance, tyrothricin, was used. Further experiments have been carried out in which gramicidin and tyrocidine were used. When 0.5 microgram of gramicidin was added to tubes containing 1 per cent suspensions of washed sheep erythrocytes, hemolysis was complete in twenty-four hours. This amount of gramicidin produced the same amount of hemolysis as did 1 microgram of the crude substance, tyrothricin. This indicates that gramicidin is more active than tyrothricin in causing hemolysis. Tyrocidine, on the other hand, is but slightly hemolytic, as indicated by the fact that hemolysis was observed in similar preparations at the end of twenty-four hours only when 40 micrograms of tyrocidine per cubic centimeter were added to the preparation.

<sup>2</sup> We are indebted to Drs. F. R. Heilman and Luther Thompson for bacterial strains used in this study.

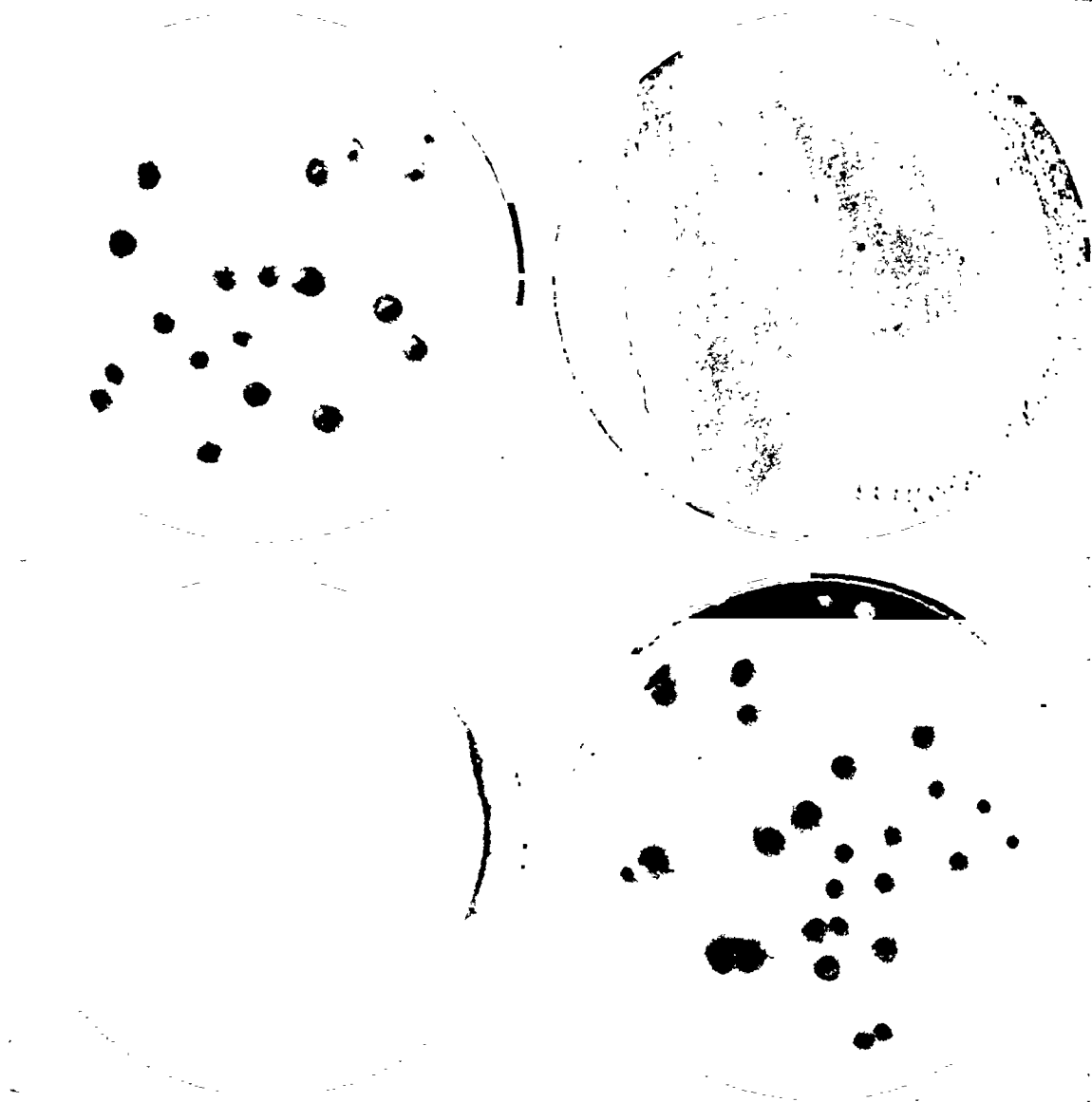


FIG. 1. TISSUE CULTURE CLOTS CONTAINING DIPLOCOCCUS PNEUMONIAE TYPE I AFTER FORTY-EIGHT HOURS' INCUBATION AT 37° C. (X 3.5)

*Left upper:* Control. *Right upper:* Clot treated with 2.5 micrograms tyrothricin per cubic centimeter media. Complete inhibition. *Left lower:* Clot treated with 1 microgram gramicidin per cubic centimeter. Complete inhibition. *Right lower:* Clot treated with 40 micrograms tyrocidine per cubic centimeter. No inhibition.

The hemolysis observed here in addition was only very slight. It seems possible that even this amount of hemolysis may be associated with the presence of minute amounts of gramicidin in the tyrocidine.

This hemolytic effect of the crude substance and its fractions was again studied in the tissue culture preparation containing 5 per cent of rabbit

erythrocytes; 0.5 microgram per cubic centimeter of both tyrothricin and gramicidin resulted in complete hemolysis at the end of twenty-four hours of incubation (Figure 3), whereas no hemolysis was observed in similar preparations in which the clot contained 30 micrograms of tyrocidine per cubic centimeter. In one of these experiments dilutions of the bactericidal substance were made in serum



so as to avoid the introduction of electrolytes into the tissue culture medium. Under these conditions hemolysis was as great as before, indicating that the addition of electrolyte did not materially influence the reaction. Experiences with tyrocidine in our laboratories would indicate that further purifi-

cation of crude tyrocidine causes progressive loss of the hemolytic activity, whereas the hemolytic activity of the gramicidin seems rather constant.

When suspensions of tyrothricin in Tyrode's solution at pH 8.0 are heated to 90° C. for ten minutes, there is a tenfold loss of hemolytic ac-

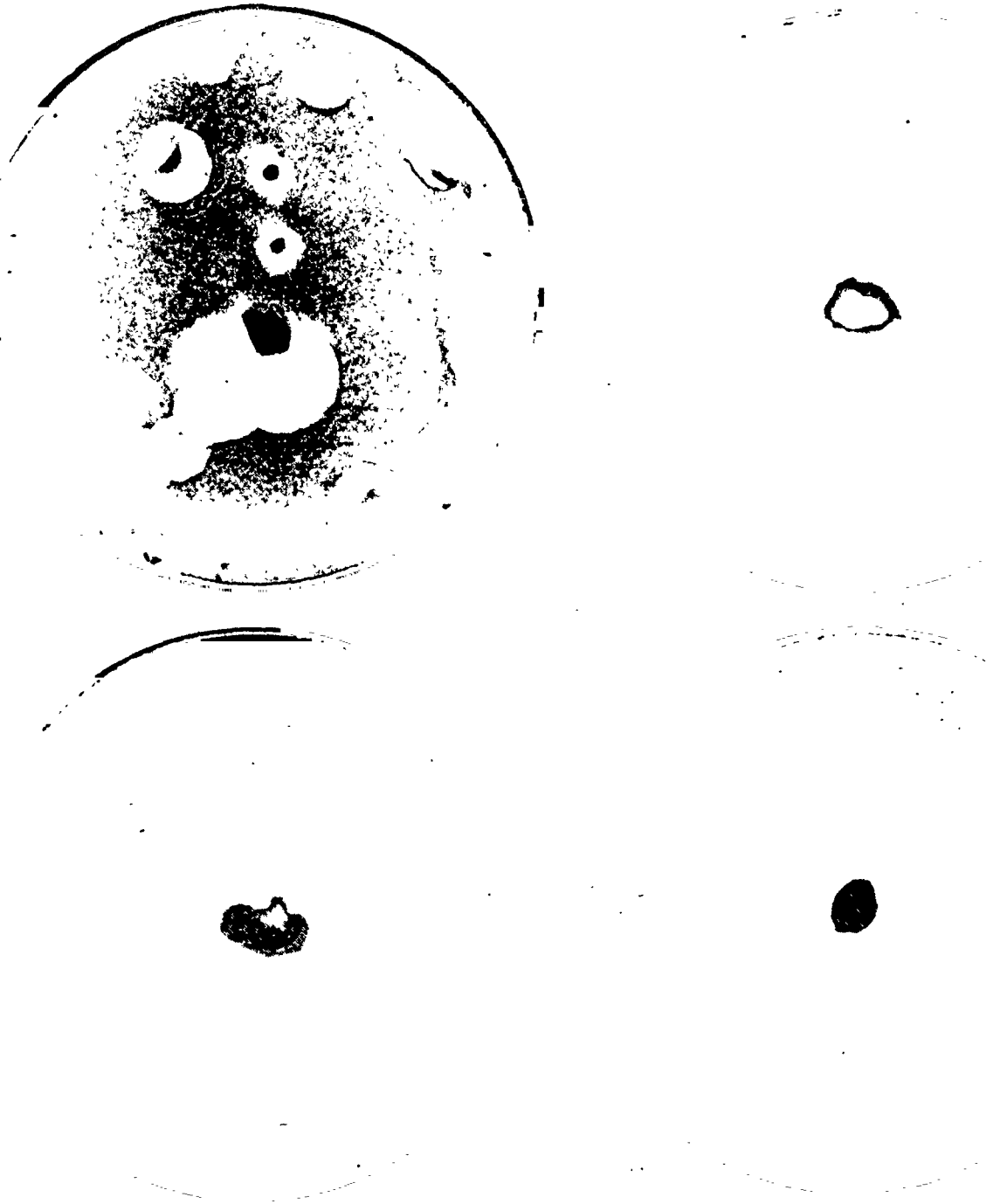


FIG. 2. TISSUE CULTURE CLOTS CONTAINING HEMOLYTIC STREPTOCOCCUS AND LYMPH NODE EXPLANTS AFTER FORTY-EIGHT HOURS' INCUBATION AT 37° C. ( $\times 3.5$ )

*Left upper:* Control. Note liquefaction of plasma around colonies. *Right upper:* Clot treated with tyrothricin 10 micrograms per cubic centimeter. Complete inhibition. *Left lower:* Clot treated with 5 micrograms gramicidin per cubic centimeter. Complete inhibition. *Right lower:* Clot treated with 100 micrograms tyrocidine per cubic centimeter. Complete inhibition.

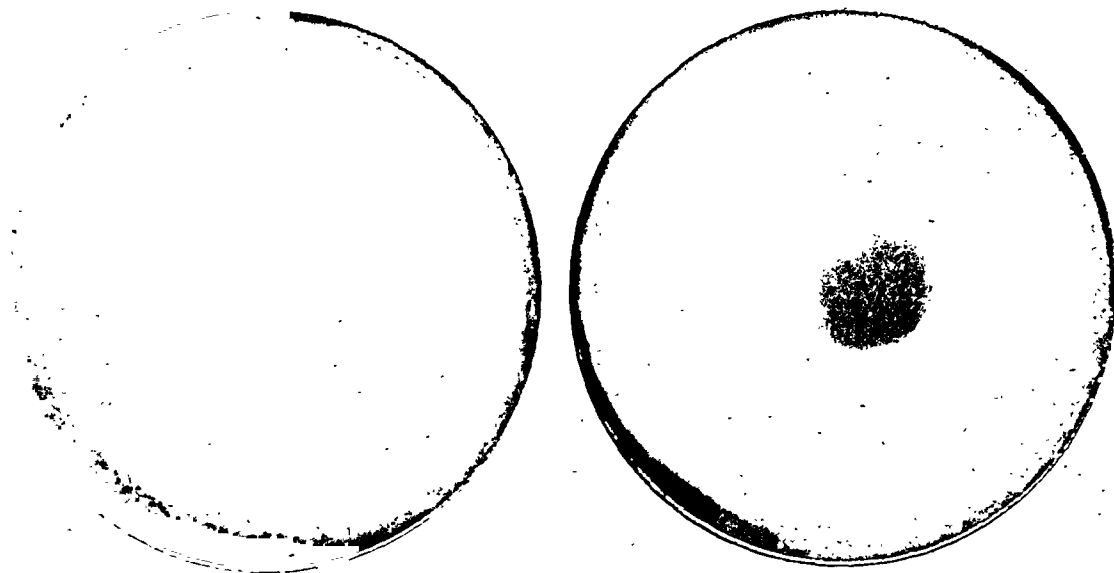


FIG. 3. TISSUE CULTURE CLOTS CONTAINING 5 PER CENT RABBIT ERYTHROCYTES INCUBATED EIGHTEEN HOURS AT 37° C. (× 3.5)

*Left:* Control. *Right:* Similar preparation to which was added 0.25 microgram gramicidin on the center of the clot. Hemolysis complete in eighteen hours

tivity in tests made on washed sheep erythrocytes. This loss of hemolytic activity does not result when tyrothricin dissolved in 95 per cent alcohol is heated to the same extent.

Suspensions of purified gramicidin were made in Tyrode's solution, the resulting suspensions having a pH of 7.8 to 8.0. These suspensions were divided into similar groups: one group was not heated; the second group was heated in a water bath at 90° C. for ten minutes; the third group was heated at 70° C. for thirty minutes; the fourth group was autoclaved at 15 pounds pressure and at 250° C. for thirty minutes. A sample of each dilution was tested in the tissue culture clot preparation containing 5 per cent of rabbit's erythrocytes; as previously described. The results of such a test are shown in Table II.

With the loss of hemolytic activity there is also a loss of bactericidal activity for bacteria growing in tissue culture. Dubos has reported that heat removes the *in vivo* activity of the bactericidal substance but does not reduce its activity *in vitro*. In this respect the behavior of gramicidin in tissue culture resembles its action *in vivo*. Pneumococci, the growth of which is regularly inhibited in tissue culture by 1 microgram of unheated gramicidin per

TABLE II

*Hemolysis of rabbit's erythrocytes in the tissue culture clot at the end of incubation at 37° C. for twenty-four hours*

	Grade of hemolysis*		
	1 µg. per cc.	10 µg. per cc.	100 µg. per cc.
Unheated gramicidin	4	4	
Gramicidin heated to 70° C. for thirty minutes	1	3	3
Gramicidin heated to 90° C. for ten minutes	0	2	3
Gramicidin autoclaved 15 lb. for thirty minutes		Very slight trace	±

\* Graded on basis of 1 to 4.

cubic centimeter, are not decreased in number by 10 micrograms of gramicidin which has been heated in Tyrode's solution at 90° C. for ten minutes. Whether or not the bactericidal property and the hemolytic property may be separated by other means will depend on the outcome of further investigation.

Studies have been made on the effect of grami-

cidin and tyrocidine on the leukocytes of whole human blood. Relatively large amounts of gramicidin and tyrocidine (50 micrograms and 100 micrograms per cubic centimeter of blood) have been added to freshly drawn heparinized human blood. Control samples to which nothing was added and samples containing suitable amounts of 95 per cent alcohol were studied at the same time. All tubes were incubated in a water bath at 37° C. for the duration of the experiment. Smears of each sample were made before the addition of the bactericidal substances and at fifteen minutes, thirty minutes, and one, one and a half, two, four, six, and eight hours thereafter. The films thus prepared were stained by the May-Grünwald technic. Dr. Watkins, one of the hematologists at the Mayo Clinic, examined the series of blood smears prepared by this method. He reported that there was no evidence of damage to the leukocytes by either gramicidin or tyrocidine. Preparations containing gramicidin showed marked lysis of erythrocytes after the first hour of incubation. Representative stained blood films are shown in Figure 4.

### *Clinical studies*

An attempt has been made to apply in a clinical way the information obtained from the experimental studies available with regard to gramicidin. No attempt has been made to use the substance where it might come in contact with the blood stream or to administer gramicidin by mouth. It was felt, however, that the substance is quite safe for local use in the treatment of conditions in which a gram-positive organism has been found on culture. The crude substance, tyrothricin, has been used entirely because we have found experimentally that its bactericidal effect is essentially the same as that of gramicidin, although it may not be as active. The tyrothricin used has been obtained from Sharp and Dohme and is the same preparation used in the experimental studies and from which gramicidin and tyrocidine were prepared. The substance may be prepared for clinical use in one of two ways. The suspension used contains 200 micrograms of tyrothricin per cubic centimeter. It may be prepared by adding 200 mgm. of tyrothricin to 1 liter of a 1.5 per cent solution of aerosol OT (ester of a sulfonated bi-

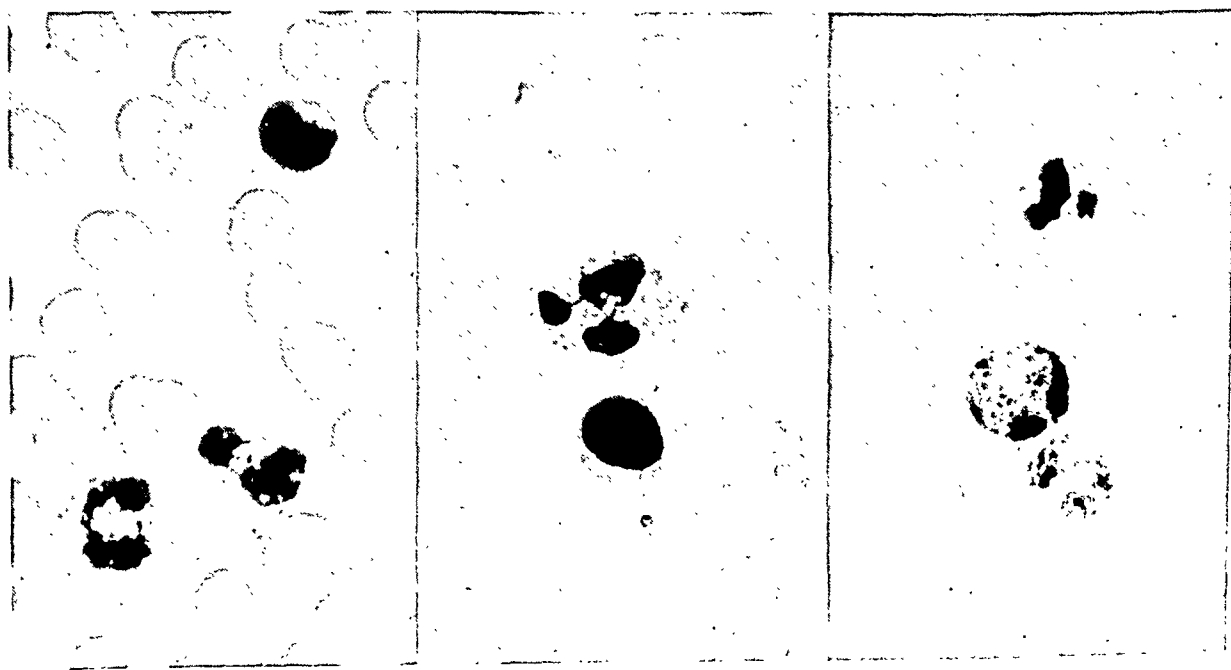


FIG. 4. WHOLE HUMAN BLOOD TREATED WITH TYROCIDINE AND GRAMICIDIN 100 MICROGRAMS PER CUBIC CENTIMETER

Smears shown made after four hours' water bath incubation at 37° C. ( $\times 1000$ ). *Left*: Photomicrograph of control blood smear. *Center*: Tyrocidine treated blood. Very minimal change in erythrocytes. Granulocytes appear normal. *Right*: Gramicidin treated blood. Marked agglutination of erythrocytes but no evidence of toxicity of granulocytes.

carboxylic acid [*di-octyl*]) in triple distilled water. The recent report by Petroff and Schain (7), in which it was stated that aerosol OT is also hemolytic, has caused us to abandon the preparation of tyrothricin in this substance for clinical use. Recently, however, we have prepared for clinical use a mixture of gramicidin in alcohol and glycerin: 200 mgm. of gramicidin are dissolved in 5 grams of alcohol and the solution is made up to 20 cc. with glycerin (U.S.P.). When 20 cc. of such a mixture are added to 1 liter of triple distilled water, the resulting solution contains 200 micrograms of tyrothricin per cubic centimeter and this is substantially isotonic with blood.

At the present time we have used the preparation in twelve cases of various types of infection. The type of infection, the gram-positive organism isolated, the amount of the preparation used locally and a summary of the results are shown in Table III. Two of the cases are of sufficient interest to warrant brief reports.

#### Report of cases

*Case 1.* A white man, twenty-nine years of age, came to the clinic January 2, 1941, because of an ulcer on the left leg. There was a history of ancient thrombophlebitis but the ulcer on the leg had appeared following an injury sustained seven months before he came to the clinic. He had had a great deal of local treatment although the ulceration was becoming larger and there was definite evidence of secondary infection. General physical examination, laboratory studies, and roentgenologic examination of the left leg did not reveal any abnormality. The patient was hospitalized on January 6, and bacterial cultures of the ulcer showed the presence of hemolytic streptococcus. A 1.5 per cent solution of aerosol containing 100 micrograms of tyrothricin per cubic centimeter was applied locally to the ulcer. By the third day there was remarkable improvement in the appearance of the ulcer. There was no evidence of any damage to the tissues. There was nothing about the patient which would suggest toxic effects; therefore, medication was continued for fifteen days. On the fifteenth day the condition of the ulcer was so good that skin grafting was carried out. Several days before, however, cultures taken from the ulcer failed to reveal any evidence of hemolytic streptococci. The pinch grafts took perfectly. Two weeks later the patient was dismissed from the hospital. When he was seen one month later the grafts had entirely healed and, except for a sponge dressing which was applied for one week, no treatment was necessary. The result was considered excellent by the consultants in the dermatology department who saw him.

*Case 2.* A man, aged thirty-four years, came to the clinic February 13, 1941, because of maxillary sinusitis

on the left side. He had been ill for approximately seven weeks and there had been a great deal of purulent drainage from the left side. Roentgen therapy and suction had been applied to the left maxillary sinus but a great deal of purulent discharge as well as pain and tenderness had continued. The pain and purulent discharge had persisted for ten days after the treatment and he had been advised to undergo surgical treatment. At the time operation on the left maxillary sinus was considered, a very acute infection developed in the right maxillary sinus which had been entirely normal until that time. From this side a culture was obtained which showed the presence of hemolytic streptococcus. An attempt was made to treat this sinus with tyrothricin. The patient was admitted to the hospital and 20 cc. of the preparation of tyrothricin (200 micrograms per cubic centimeter) were introduced into the right maxillary sinus by means of the displacement technic; 20 cc. of this preparation were administered in this manner every three hours for two days. At the end of twenty-four hours the pain was markedly relieved and the patient was afebrile. The drainage decreased accordingly, and the patient's recovery was striking and uneventful. Surgical treatment on the left side was then carried out three days after the treatment of the right sinus with tyrothricin. A persistent sinusitis did not develop on the right side and no further treatment was necessary. Cultures from the nose revealed only gram-negative bacilli, and streptococci were no longer recovered from nasal cultures. A total of 320 cc. of the preparation was used.

#### COMMENT

The cases reported indicate that the results were somewhat irregular. This is due in part to the fact that in some instances inadequate amounts of tyrothricin were used in the beginning. On the other hand, there is some difference in the response of the conditions treated with relation to the pathogenic organism isolated. Infections caused by hemolytic streptococci seem to respond most readily, and the staphylococcal infections appear to be the most stubborn when similar amounts of tyrothricin are used. This is especially interesting in view of the fact that *Staphylococcus aureus* experimentally is much more resistant than most of the strains of streptococci studied by us in the tissue culture. It would appear that the most striking clinical results were obtained in the treatment of definite ulceration. This is especially true of the infected stasis ulcers. The few conditions in which infection occurred in a cavity into which adequate amounts of the substance could be placed also responded satisfactorily. Dermatitis, on the other hand, with the possible exception of

TABLE III  
Results of local use of tyrothricin

Case	Diagnosis	Organisms	Solution of tyrothricin*		Response	Additional treatment
			Concentration	Total amount administered		
1	Ulcer of leg	Hemolytic streptococcus	µg. per cc. 100†	cc. 3,000	Ulcer became clean; culture became negative in one week	Pinch grafts on fifteenth day; all grafts took
2	Acute maxillary sinusitis	Hemolytic streptococcus	200	320	Striking clinical improvement; cultures became negative	None
3	Postoperative empyema	Staphylococcus aureus and hemolytic streptococcus	10	150	None; patient would not continue treatment	Sulfanilamide applied locally
4	Dermatitis of hands and feet	Hemolytic streptococcus	200	1,000	Good initial response; purulent drainage increased subsequently; tyrothricin discontinued	Sulfathiazole ointment; dressings of 0.5 per cent solution of aluminum subacetate
5	Bilateral otitis media	Non-hemolytic streptococcus	10	8	None; treatment inadequate	Abscess of right ear was drained
6	Extensive hidrosadenitis suppurativa of axilla; ulcer of leg	Hemolytic streptococcus	200	12,000	Very striking; drainage decreased, cultures became negative and lesions healed	Surgical excision of undermined portions of skin
7	Eczematoid dermatitis of hands and feet	Hemolytic streptococcus and gram-positive bacillus	200	3,000	Excellent, cultures became negative	Roentgen therapy
8	Eczematoid dermatitis of hands and feet	Hemolytic streptococcus and Staphylococcus aureus	200	500	Better on right side, but not striking	Left hand and foot treated with sulfathiazole ointment but not with tyrothricin
9	Stasis ulcer	Hemolytic streptococcus and Staphylococcus aureus	10	600	Ulcer became clean rapidly but never healed completely. Staphylococcus aureus persisted. Treatment probably inadequate	Solution of potassium permanganate used locally
10	Eczematoid dermatitis	Hemolytic streptococcus	200	1,000 (?)	Culture became negative but additional treatment was necessary	Wet dressings of aluminum subacetate; colloidal baths
11	Stasis ulcer	Staphylococcus aureus	200	500	Poor	Skin grafts
12	Very persistent cystitis	Streptococcus faecalis and Staphylococcus aureus	200 to 400	1,500	Excellent; first negative cultures in one year	Additional treatment none

\* In triple distilled water unless stated otherwise.

† In 1.5 per cent solution of aerosol OT.

the dermatitis in Case 7 (Table III), did not respond well to this form of treatment, although in many instances the pathogenic organisms frequently disappeared. Additional therapy, however, almost always must be employed.

#### SUMMARY

The bactericidal effect of tyrothricin, tyrocidine, and gramicidin has been studied by using the tissue culture technic. Gramicidin is more effective than tyrocidine against most of the gram-positive bacteria studied. The tissue culture method permits not only the study of the bactericidal effect of these substances but at the same time an opportunity is afforded to observe the possible effects of the substance on bacteria growing in the presence of tissue fragments, serum and tissue extract. These conditions approach the circumstances which obtain *in vivo*.

Tyrothricin has a powerful hemolytic action on erythrocytes *in vitro*. The hemolytic effect of tyrothricin is due to the presence of gramicidin. When tyrothricin or gramicidin is heated in an aqueous suspension there is loss of hemolytic and bactericidal activity. Tyrocidine does not appear to be very hemolytic.

Neither gramicidin nor tyrocidine appears to produce any marked toxic effect upon the leukocytic elements of the human blood in amounts up to 100 micrograms per cubic centimeter over a period of eight hours.

Tyrothricin has been used locally in twelve cases of various types of infections in which gram-positive bacteria were present. Marked beneficial

effect was noted in most cases in which the substance was used. No demonstrable damaging effects have been noted on the tissues. On the other hand, the healing of wounds has appeared to be considerably benefited in some instances. No evidence of toxicity has been observed following the use of the substance in the manner described. The hemolytic effect of gramicidin is great enough in the presence of constituents of the blood to render inadvisable the clinical use of this substance in any way except locally or perhaps to irrigate infected cavities which do not communicate with the blood stream.

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# ACUTE HEMOLYTIC ANEMIA FROM THE SULFONAMIDES

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Within the past year we have seen 9 cases of acute hemolytic anemia in the course of sulfanilamide or sulfapyridine therapy, with 6 deaths. In the hope of explaining the mechanism of this hemolysis, the laboratory studies which form the basis of this report were conducted in 4 cases. The hemoglobin derivatives in serum, erythrocytes and urine were examined by the recording photoelectric spectrophotometer. The high degree of accuracy obtainable with this objective method permitted comparison of the hemoglobin derivatives in the serum derived from destroyed red cells with those in the cells that remained intact. The new blood pigment, methemalbumin, recently described by Fairley (1), was detected in the plasma. The findings are reported and interpreted in relation to the mechanisms of hemolysis, hemoglobinuria and hemolytic shock.

## *Clinical data (cases listed in order of increasing severity)*

*Case 1, P. P.* (Number 459109), negro, age 32, was under treatment for infected bite of the hand. The Wassermann reaction was positive. He received 24 grams of sulfanilamide in 4 days. On the morning of the fifth day, intense hemoglobinuria occurred and jaundice was noted. The hemoglobin concentration of the blood, not measured heretofore, dropped from 75 per cent (Sahli) on the morning of the fifth day to 60 per cent at 4 p.m. At this time the highly colored specimens of plasma and urine to be discussed below were obtained; his icterus index was 38 and his blood urea N 16. The blood picture was that of hemolytic anemia, with 11 per cent reticulocytes; there was no sickling. The hemoglobinuria lasted about 36 hours. The urine showed occasional granular casts but no red blood cells. There were no further symptoms; sulfanilamide therapy was stopped, the jaundice continued for 4 days and the patient recovered.

*Case 2, J. M.* (Number 454755), Italian, male, age 43, was admitted with severe erysipelas of the face 48 hours after puncture of a sinus for suppurative ethmoiditis. He received a total of 12 grams of sulfanilamide during 2 days. On the second day he was observed to be jaundiced (icteric index 33) and there was shock tenderness over

the liver. Blood hemoglobin was 104 per cent (Sahli); the blood culture was sterile, and the blood sulfanilamide level was 4.0 mgm. per cent. On the third day, although the erysipelas appeared to be controlled, the temperature rose to 103.6° F. The urine at this time contained bilirubin but no hemoglobin. On the fourth day, the jaundice appeared deeper and the blood hemoglobin had fallen to 75 per cent. On the fifth day a paroxysm of hemoglobinuria occurred accompanied by severe nausea, vomiting, abdominal and lumbar pain and tenderness. The urine showed large amounts of hemoglobin but no red blood cells. The blood hemoglobin fell to 34 per cent with 1.8 million red blood cells; the cell volume was 16 per cent. The white blood cells rose from 16,000 to 25,000. The platelets were normal. Blood smears showed 9 per cent myelocytes, 8 per cent normoblasts, and 8 per cent reticulocytes. The icteric index rose to 108, blood cholesterol was 205 mgm. per cent with cholesterol esters reduced to 70 mgm. per cent. The patient appeared very ill but was not in collapse. Transfusion of 1 liter of blood was given and, in order to induce excretion of an alkaline urine, 4000 cc. of Hartman's solution and glucose and saline solutions were administered daily. As a result there was a copious diuresis—8000 cc. during the 2 days of hemoglobinuria. On the sixth day, the patient was still in great discomfort; the icteric index was 90, hemoglobinuria was still present though less marked, and another transfusion of 500 cc. of blood was given. On the seventh day, the patient's condition was better; the hemoglobinuria ceased, jaundice and bilirubinuria were still present. From this point on the patient, after two additional blood transfusions, gradually recovered.

*Case 3, B. E.* (Number 462878), a physician, age 39, had an infection at the tip of his nose. He took a few doses of sulfanilamide and the local condition cleared up. After one or two days, however, he continued to have some fever and, as some râles were heard at the base of the left lung, he took sulfapyridine, a total of only 11 grams in 2 days. At the end of this time he suddenly became very acutely ill with high fever, extreme pallor, profound jaundice and great prostration. Hemoglobin was noted to have fallen from 80 to 40 per cent and his white blood count rose from 12,000 to 50,000. On admission to the hospital his blood pressure was 128/70. His spleen and liver were greatly enlarged. His urine was Burgundy red; microscopically it showed casts and occasional red blood cells.

He was given a transfusion of 500 cc. at once and 600 cc. the next day; his hemoglobin rose to 67 per cent. At this time his blood urea was found to be 69 mgm. per

<sup>1</sup> Aided in part by the Dazian Foundation.



cent and his blood carbon dioxide combining power was 33 volumes per cent. The blood sulfapyridine 24 hours after sulfapyridine therapy had been stopped was still 7.4 mgm. per cent. There was a moderate degree of oliguria, although there was never complete suppression of urine; during the 2 days before his death he secreted 1650 cc. of urine. There was a steadily progressive rise of temperature until 107° F. was reached. This was accompanied by increasing shock. A sample of his blood plasma taken the last day of his life (the third day after the onset of the hemolytic anemia) still was nearly black.

Postmortem examination showed, besides a bronchopneumonia, the characteristic findings of acute hemolysis: icterus, marked edema of the liver with striking enlargement of the Kupffer cells which contained nuclear debris and red blood cells. The liver cells themselves were not diffusely damaged, but scattered small foci of four or five necrotic liver cells were present; these might have been caused by small capillary blockages due to Kupffer cell proliferation (Dr. Klemperer). The spleen weighed 480 grams; it showed a tremendous degree of active hyperemia and for practical purposes was indistinguishable from a spleen of hemolytic icterus. The kidneys showed an immense number of hemoglobin casts, chiefly in the deeper tubules and Henle's loops.

*Case 4, I. L.*, the father of a doctor, a man age 63, never seriously ill before, developed bronchial pneumonia. No sputum was obtained; blood culture was negative. He was given 5 grams of sulfanilamide the first day and then 12 grams of sulfapyridine during the following 2 days. Because of the onset of severe vomiting, cyanosis, and weakness, the sulfapyridine was stopped. Twenty-four hours later it was noted that the urine was of mahogany color and there was severe deep lumbar pain. At this time the pulse rate and the blood pressure, which had been normal, underwent a sudden change; the pulse rose to 138 and the blood pressure became unobtainable; the patient was obviously in shock. The blood showed a hemoglobin of 50 per cent, with red cell count of only 1,000,000. This discrepancy was explained, however, when the blood plasma was obtained, since it was almost black. The white blood count was 32,000, the platelets 400,000; the blood smear showed 10 per cent myelocytes and 13 nucleated red blood cells per 100 white blood cells. The urine microscopically showed no red blood cells but much amorphous deposit. Urine secretion gradually diminished during 24 hours until it ceased altogether. The total amount of urine in the last 2 days of life was approximately 1500 cc. The jaundice first noticed on the fourth day after beginning of medication became deeper and the urea nitrogen of the blood rose to 42 mgm. per 100 cc. In spite of three transfusions of 500 cc. each, which brought the red cell count to 2,000,000 and the blood pressure to 134/84, the pallor and collapse continued and the patient died on the fifth day after the onset of his pneumonia, about 48 hours after onset of the hemolytic shock.

## METHODS

Urine and plasma<sup>2</sup> were examined directly, diluting when necessary for accuracy. Red cells from citrated blood were washed three times with physiological salt solution, suspended in M/100 phosphate buffer, pH 6.9, and laked with saponin. For identification and definite detection of small amounts of abnormal pigments, ordinary spectroscopy with recognition of absorption bands is inadequate. Only careful spectrophotometric determination of light absorption at each wavelength followed by observations of changes in light absorption after the addition of various chemicals gives reliability to the findings.

For this purpose two instruments were used: (1) a photoelectric spectrophotometer with a Lange galvanometer,<sup>3</sup> and (2) the Hardy recording spectrophotometer.<sup>4</sup> Transmission curves of all solutions were made before and after addition of neutral 5 per cent sodium cyanide solution. In the figures, only the significant parts of the curves are shown.

Methemoglobin was estimated by the change in optical density at wavelength 630 mμ by the formula:<sup>5</sup>

$$\frac{\text{Optical density } \lambda 630 \text{ m}\mu \text{ (initial)}}{\text{Optical density } \lambda 630 \text{ m}\mu \text{ (after CN)}} = 1.8 = \text{grams methemoglobin per 100 cc.}$$

Total hemoglobin was measured in those specimens containing only hemoglobin and methemoglobin by the

<sup>2</sup> Specimens were examined as soon as obtained. In some instances urines were examined after standing overnight. Controls, however, of hemoglobin added to urine showed that under these conditions only a little of the hemoglobin is converted into methemoglobin; serum when kept in the icebox remains unchanged overnight. On account particularly of the marked effect of pH on the absorption band of methemoglobin (9) it is important to note that all solutions except some sera were diluted before spectroscopy with M/100 phosphate buffer solution of pH 6.9. When sera were used undiluted they were fresh and it was believed they were adequately buffered. Red cells were diluted one hundred times; urines were diluted five to twenty-five times.

<sup>3</sup> We are profoundly indebted to Dr. Bruce Hogg of the Department of Surgery, College of Physicians and Surgeons, Columbia University, who set up the apparatus (2) and performed some of the first tests for us (3).

<sup>4</sup> This is an entirely automatic instrument which records a continuous curve at all visible wavelengths with no personal equation whatever (4). For its use we wish to thank the Electrical Testing Co., York Ave., New York, and the American Cyanamid Co., Stamford, Conn.

<sup>5</sup> This formula was obtained by previous studies (5) using the Hardy spectrophotometer and differs from that of Drabkin and Austin (9) in that the maximum absorption of methemoglobin is located at λ630 mμ instead of λ634 mμ (See also Michel (6).)

TABLE I

*The approximate distribution of blood pigments in the surviving red cells, serum, and urine of 4 cases of acute hemolytic anemia*

	Per cent distribution of total pigments	Total pigment	
	0                      50                      100		grams per 100 cc.
Case 1		Red blood cells	
		Serum	0.39
		Urine	0.67
Case 2		Red blood cells	
		Serum	0.69
		Urine	0.38
Case 3		Red blood cells	
		Serum	0.34
		Urine	0.25
Case 4 *		Red blood cells *	
		Serum	1.66†
		Urine	0.87
<div style="display: flex; justify-content: space-around; align-items: center;"><div style="border: 1px solid black; width: 20px; height: 10px; display: inline-block;"></div> = HEMOGLOBIN    <div style="background-color: black; width: 20px; height: 10px; display: inline-block;"></div> = METHEMOGLOBIN</div> <div style="display: flex; justify-content: center; align-items: center; margin-top: 5px;"><div style="background: repeating-linear-gradient(45deg, transparent, transparent 2px, black 2px, black 4px); width: 20px; height: 10px; display: inline-block;"></div> = METHEMALBUMIN</div>			

\* Red blood cells of this patient contained 4 per cent sulfhemoglobin.

† By iron method 2.00 grams per cent.

method of Stadie (7). This converts oxyhemoglobin to methemoglobin by means of potassium ferri-cyanide and then changes the methemoglobin to cyanmethemoglobin by means of cyanide. Specimens of plasma containing sulfhemoglobin or methemalbumin (which does not combine with cyanide) cannot be measured in this way, and in the fourth case the total was measured by the alpha alpha' dipyrindyl iron method (8).

The method used for determination of the new pigment methemalbumin in the serum will be discussed under the caption "Results—Sera."

## RESULTS

**Red blood cells.** In the figures are shown the relevant portions of the spectrophotometric curves of the washed, laked erythrocytes of the 4 patients. The curves of the first 2 are smooth with no indentation or band to indicate any abnormal pigment. Upon addition of cyanide, however, there is a small decrease in the optical density, indicating the presence of a proportionately small amount of methemoglobin. On calculation the

amounts of methemoglobin in these cases (Table I) are found to be small enough to escape detection by ordinary methods. The third and fourth cases, however, which were fatal, showed a larger amount of methemoglobin with an absorption band at  $\lambda 630 \text{ m}\mu$  which was dispersed with cyanide.

Unfortunately, at the time we knew of no such combined chemical-optical method for the quantitative measure of sulfhemoglobin.<sup>6</sup> But it may be pointed out that, since the absorption band of sulfhemoglobin at  $\lambda 620 \text{ m}\mu$  is nearly three times as intense as that of methemoglobin at  $\lambda 630 \text{ m}\mu$ , similar small amounts of sulfhemoglobin if present would have produced a detectable indentation of the transmission curve after cyanide at  $\lambda 620 \text{ m}\mu$ . Such a very small deviation is actually shown in Case 4. It may be stated, therefore,

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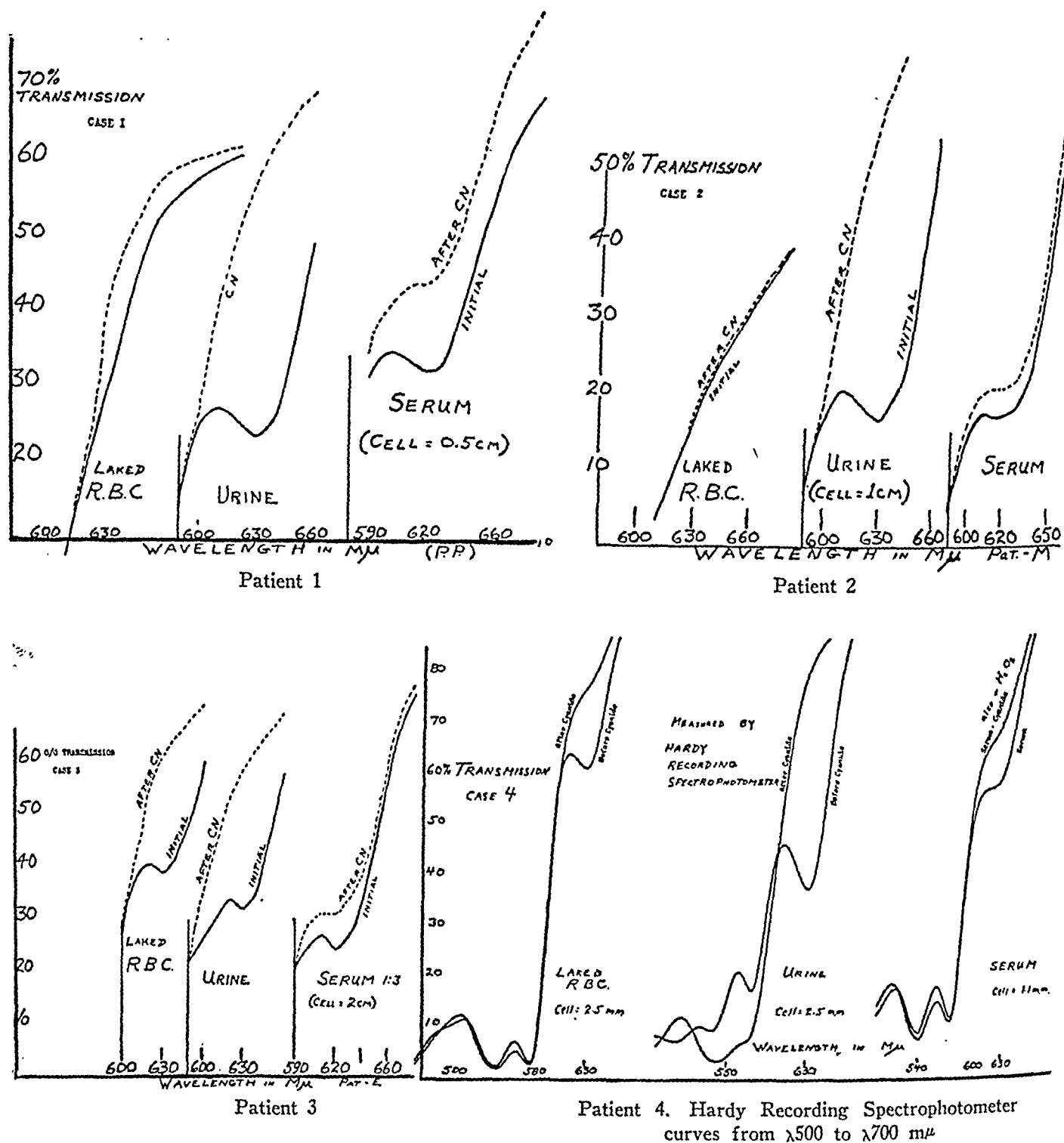


FIG. 1. SPECTROPHOTOMETRIC CURVES OF LAKED RED BLOOD CELLS, URINE, AND SERUM OF 4 CASES OF ACUTE HEMOLYTIC ANEMIA

Ordinates = per cent transmission  
Abscissae = wavelengths

———— = initial curve  
----- = curve after cyanide

that (except for Case 4) erythrocytes of these patients contain no detectable sulfhemoglobin.

*Urines.* The spectrophotometric curves of the urines of these patients are different from those of the red blood cells in one important regard. There is a sharp peak of light absorption pre-

cisely at  $\lambda 630$  m $\mu$ . Following the addition of cyanide, this peak disappears completely and the resulting curve is quite smooth. This is characteristic of large amounts of methemoglobin admixed with hemoglobin; and, on calculation, the percentages of methemoglobin range from 44 to

83 per cent of the total blood pigments present in the urine (Table I). The location of the sharp peak at  $\lambda 630 \text{ m}\mu$  and the disappearance of this absorption band, leaving a smooth curve after the addition of cyanide, prove the absence of sulfhemoglobin and methemalbumin from the urine.

*Sera.* The sera of these patients are quite different. Instead of a smooth curve or a sharp absorption peak, they show a broad band of absorption extending from  $\lambda 610 \text{ m}\mu$  to  $\lambda 640 \text{ m}\mu$ . On analysis by the addition of cyanide, the curve does not become smoothed out as in the case of the urine. The absorption in the region of the red is decreased and measurement of the density change at  $\lambda 630 \text{ m}\mu$  permits computation of the amount of methemoglobin present (Table I). The cyanide-resistant broad band centered at  $\lambda 620 \text{ m}\mu$  is indicative of the simultaneous presence of still another pigment. Since sulfhemoglobin has its characteristic absorption at  $\lambda 620 \text{ m}\mu$ , we at first attempted to ascribe this absorption to sulfhemoglobin. Computations using Austin and Drabkin's absorption coefficients (9), however, did not fit the observed data. We then became aware of Fairley's discovery in the plasma of blackwater fever of a new pigment, methemalbumin, which also has an absorption band in this region (1). The absorption band of this pigment, like that of sulfhemoglobin, is not obliterated by cyanide. These pigments can be differentiated from each other, however, by the addition of  $\text{H}_2\text{O}_2$  which causes the disappearance of the band of sulfhemoglobin at  $\lambda 620 \text{ m}\mu$  but does not disturb the band of methemalbumin.

The unique finding in all the sera was the presence of a broad band about  $\lambda 620 \text{ m}\mu$  not obliterated by cyanide. In the fourth case, this was further tested with 3 per cent hydrogen peroxide but no change in the light absorption occurred. This proved the presence of methemalbumin and the absence of sulfhemoglobin.

In order to determine methemalbumin quantitatively, this pigment was prepared by incubating sterile normal plasma with hemoglobin at  $37^\circ \text{C}$ . for 4 weeks. The absorption spectrum was determined as described elsewhere (10). With the absorption coefficients so obtained, the amounts of hemoglobin and of methemalbumin could then be calculated from the observed data at the peak

wavelengths  $\lambda 540 \text{ m}\mu$  and  $\lambda 620 \text{ m}\mu$  by solving two simultaneous equations<sup>7</sup> of the following general form:

Optical density observed minus absorption due to methemoglobin<sup>8</sup> = absorption due to hemoglobin plus absorption due to methemalbumin.

$$\begin{aligned} \text{O.D.}_{620} - \text{concentration MHb} \times \text{coefficient} & \\ & \text{MHb}_{620} \\ = \text{concentration Hb} \times \text{coefficient Hb}_{620} + \text{con-} & \\ \text{centration MetAlb} & \times \text{coefficient MetAlb}_{620}. \end{aligned}$$

The total blood pigments would naturally be the sum of the concentrations of methemoglobin, hemoglobin and methemalbumin (Table I). In the fourth case this was checked independently by iron determination and a reasonably close correspondence was obtained, the difference presumably representing iron already freed in bilirubin formation.

The results of these examinations compiled in Table I indicate that, after an acute hemolytic episode, the surviving red blood cells are essentially normal or contain but little methemoglobin, far less than plasma and urine. In addition, the plasma contains a third pigment, methemalbumin, which is readily formed in the plasma from extracorporeal hemoglobin.

In Table II are shown the total amount of hemoglobin liberated (E) and the amount liberated in relation to patients' weight (G), the plasma level of hemoglobin at a variable number of hours after the onset of hemolysis (H), the amount of hemoglobin remaining in the circulating plasma at this time (I), the total hemoglobin excreted (J), and the relation of this to the hemoglobin

<sup>7</sup> This procedure has been used satisfactorily heretofore, e.g., (5, 10, 11). The validity of this method might be questioned in dealing with mixtures that might contain other unrecognized pigments. Lemberg (12) has described pigments formed by the disintegration of hemoglobin but their characteristics are not such as to be confused with methemalbumin. In the serum of Case 3, in which there was practically no hemoglobin remaining, the concentration of methemalbumin was calculated directly from the residual absorption at  $\lambda 620 \text{ m}\mu$  after cyanide (10). The concentration found was 0.258 gram per cent as against 0.263 gram per cent by simultaneous equations.

<sup>8</sup> Methemoglobin determined independently by change in density at  $630 \text{ m}\mu$  after addition of cyanide.

TABLE II  
 Removal of hemoglobin liberated by hemolysis

A  Case number	B		C  Hemo- globin* liber- ated	D  Blood volume†	E  Total hemo- globin liber- ated (C×D)	F  Patient's weight	G  Hemo- globin liberated E/F	H  Plasma level of total pigment	I  Hemo- globin remaining in plasma at time levels were taken (D×H×10)	J	K
	Red blood cell counts									Hemoglobinuria	
	Hemolysis									Total excreted	Per cent of total hemo- globin liberated (J/E×100)
	Before	After									
	millions		grams per liter of blood	liters	grams	kgm.	grams per kilo of weight	grams per 100 cc.	grams	grams	
1	5.0	3.0	62	7.9	490	90	5.5	0.39(0.22)‡	23	14	3
2	5.5	1.8	115	6.6	760	75	10.1	0.69(0.49)	39	24	3
3	5.0	2.0	94	6.2	583	70	8.1	0.34(0.08)	17	5	1
4	4.5	1.0	109	7.0	763	80	9.5	1.66(1.22)	104	12	2
48A					6.2		0.102	0.25		0	—
48B					8.		0.24	0.32		0.4	5
50					7.3		0.10	0.25		0.6	8

\* Estimated on basis of 5,000,000 red blood cells per cmm. and corresponds to 15.6 grams hemoglobin per 100 cc. blood (Haden).

† Calculated as 8.8 per cent patient's weight.

‡ The figures in parenthesis ( ) represent the excretable pigments hemoglobin and methemoglobin.

liberated (K). Since plasma volumes could not be measured, the estimations in columns C, D, E, G, I, K are, of course, only approximate. For comparison, similar data are added for 3 cases selected from a previous study (13) in which measured amounts of hemoglobin were injected intravenously in normal human beings. Here E represents the grams of hemoglobin injected, and the plasma levels (H) are those noted at once after injection.

#### DISCUSSION

While the objective of finding the cause of the hemolysis was not attained, the observations permit certain deductions as to (1) mechanism of hemolysis, (2) disposal of hemoglobin and (3) cause of death.

##### (1) Mechanism of hemolysis

Fairley's demonstration (1) that methemalbumin is formed *in vitro* and *in vivo* from extracorporeal blood pigments in human serum is evidence that the methemalbumin in the present case was secondary to the hemolysis and was not directly mediated by the sulfonamides. It apparently does not play a rôle in the mechanism of

hemolysis. Sulfhemoglobin may also be excluded since none was found (except 4 per cent in the cells of Case 4).

The occurrence of large proportions of methemoglobin in the serum with less in the intact red blood cells suggests the possibility that it was the red blood cells containing methemoglobin which underwent hemolysis.<sup>9</sup> This might indicate that formation of methemoglobin in the red blood cells was an etiological factor. Inasmuch as methemoglobin is formed from hemoglobin only by oxidizing agents (*e.g.*, ferricyanide), the uniform occurrence of some methemoglobin in the red cells of all patients treated with the sulfonamide drugs (3, 14) is excellent evidence that the body produces oxidizing agents from these drugs (15). Furthermore, one such oxidant, hydroxylaminobenzene sulfonamide, unlike ferricyanide, can penetrate the erythrocyte membrane and is detectable in the urine during sulfanilamide therapy (16). The contrast between the frequency of methemoglobin in the red blood cells and the infrequency of hemolytic anemia shows that, al-

<sup>9</sup> It is not certain, however, how much of the methemoglobin in the serum was present in the red blood cells before hemolysis; some may have been formed after hemolysis by the unknown oxidant.

though oxidizing agents are usually formed, they *per se* are not hemolytic. Furthermore, if the usual oxidation products were hemolytic, one would of necessity expect that hemolysis would parallel methemoglobin formation. This is not the case. Most patients receiving huge doses over long periods of time with correspondingly large amounts of methemoglobin in their cells (up to 40 per cent in man (14), 75 per cent in monkeys (17)), do not develop hemolysis. On the other hand, when hemolytic anemia occurs, it appears early—within 2 to 5 days from the start of administration, and after only moderate doses of the drug (only 12 grams in one of the present cases). Accordingly, it is possible that certain individuals form unusual oxidation products which are hemolytic and which are present in or affect chiefly the red cells containing methemoglobin. For example, the nitro compound representing a further stage of oxidation of the amino group of sulfanilamide than the hydroxylamine is more toxic as well as more bacteriostatic (18). Presumably, this peculiarity is permanent for the individual, for in 3 out of 4 patients who once suffered acute hemolytic anemia from sulfanilamide, readministration of the drug a year later again induced this syndrome and on the identical day (19).

It has been stated that a "transient but striking increase of fragility to sodium chloride in 5 cases of severe sulfanilamide hemolytic anemia" was a preliminary stage in hemolysis (20). The absence of increased fragility, on the other hand, in 3 cases studied after the hemolysis had been completed (21) is explicable by our finding that the red blood cells not hemolyzed were relatively normal, *i.e.*, free of methemoglobin; this suggests that the fragility reported only lasts until the already damaged red blood cells have been laked.

It is possible, also, in view of the peculiar timing and small inciting dosage, that this syndrome is the result of drug allergy. Landsteiner (22) has shown that antibodies may be produced after sensitization with many drugs chemically linked to proteins, and this has recently been carried out with the sulfonamides (23). None of the other drugs known to cause drug allergy, however, produces hemolytic anemia. Furthermore, drug fever and skin rashes, complications of sulfonamide

therapy more readily attributable to drug allergy, are not associated with hemolytic anemia.

## (2) *Disposal of hemoglobin; hemoglobinuria, bilirubin formation*

In the previous study of the renal threshold in man (13), hemoglobin levels of 0.32 gram per 100 cc. were obtained immediately after intravenous injection of 8 grams of hemoglobin. Removal of the hemoglobin from the circulation was very rapid, being complete in all cases at 12 hours. In the hemolytic anemia cases the amounts of hemoglobin liberated were 50 to 100 times greater (495 to 763 grams). If the acute hemolysis occurred at one time, the initial levels of hemoglobin would have ranged around 10 grams per 100 cc. of plasma. The actual levels, 1.66 to 0.34 gram per cent found 12 to 48 hours later, show how rapid must have been the removal of the huge amounts of hemoglobin.

A surprisingly small proportion of all this hemoglobin was excreted<sup>10</sup>; in the experiments, never more than 8 per cent of the amount injected; in the hemolysis cases, not over 4 per cent of the amount liberated. This does not point to a renal threshold (25) with excretion of all hemoglobin above some given plasma level.

What happens to the retained hemoglobin? Some is obviously transformed almost at once to bilirubin. The intense and prompt jaundice<sup>11</sup> shown by all the patients may reasonably be attributed to overloading of the liver by the bilirubin produced suddenly from several hundred grams of hemoglobin. The normal liver excretes in 24 hours an amount of bilirubin derivable from destruction of about 12½ grams of hemoglobin (27) but is known to have considerable reserve capacity (28). The previous experimental injection of amounts up to 8 grams of hemoglobin produced scarcely measurable increases in blood bilirubin (13); injections of amounts up to 16 grams produced slight bilirubinemia (29). That the jaundice is due to the excess of bilirubin rather

<sup>10</sup> Many cases of hemolytic anemia have gross hematemesis and melena (24). It is not known whether this represents excretion of hemoglobin or actual hemorrhage.

<sup>11</sup> Every 100 grams of hemoglobin may be converted to 4 grams of bilirubin. Certain tissues absorb large amounts of bilirubin in excess of their water content (26).



than liver damage is shown by the minor liver changes at autopsy (our Case 3 and (30)) and by the prompt disappearance of jaundice in those cases which recover. This contrasts with the marked liver damage in the non-hemolytic type of sulfonamide jaundice (31).

### (3) Cause of death

Renal blockage due to obstruction of the tubules by precipitated hemoglobin is frequently stated to be the cause of death in hemoglobinuria (32). De Navasquez (33) has recently restudied this question. His analysis of human and animal material does not support the view that simple mechanical blockage of the renal tubules is an adequate explanation. Suppression of urine occurred in several of our cases (not those detailed in this report) and one of them had anuria for 24 hours, but none of them died of it. When death follows from renal insufficiency in hemoglobinuria, it is caused by uremia and occurs at a much later date.

Anuria or oliguria can be caused by shock, and our severe cases were in shock. The cause of shock from hemolysis is unknown. Attempts to detect a high degree of toxicity in dissolved red cells themselves have failed.<sup>12</sup> Laked red cells are relatively innocuous (although most of the experiments on this point have been done with doses proportionately far smaller than the huge mass of cells dissolved in the present cases). Potassium from dissolved red cells has been suggested as the toxic agent (34) but the symptoms of potassium poisoning are different from those of hemolytic shock.

In experimental hemoglobinemia (13), and in the present cases (Table II), the products of hemolysis disappear with extraordinary rapidity from the circulation. The red cells normally constitute 45 per cent of the blood volume. The destruction and removal of  $\frac{2}{3}$  of the original number of red cells with the resulting 30 per cent reduction in blood volume might be a contributory factor in producing shock.

Reviewing the course of the severest cases, it is apparent that transfusion was of no great benefit,

possibly because of further hemolysis of the new blood. Plasma transfusion would be free of this danger.

### SUMMARY

The acute hemolytic anemia of sulfanilamide and sulfapyridine therapy was studied in 4 cases, two of them fatal.

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The relation of methemoglobin and hemolysis to oxidation products of the sulfonamides is discussed.

Approximate calculation was made of the amounts of hemoglobin liberated in these explosive hemoglobinemias. Comparison with the small amounts left in the circulation after 12 to 48 hours, and the still smaller amounts (4 per cent or less) excreted by the kidneys, shows that the body has means of removing rapidly from the plasma some 500 to 700 grams of hemoglobin without the aid of renal excretion.

Shock was a prominent symptom most marked in the fatal cases. The hemolyzed cells in these cases represented about 30 per cent of the total blood volume. This much reduction in blood volume following the demonstrated rapid removal of the products of hemolysis from the circulation may be a factor in the explanation of hemolytic shock.

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# SPECTROPHOTOMETRY OF FAIRLEY'S NEW BLOOD PIGMENT, METHEMALBUMIN

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(Received for publication April 16, 1941)

In the course of the study of acute hemolytic anemia and hemoglobinuria during administration of sulfonamide drugs (1), the patients' sera were found to contain a pigment which, like methemoglobin and sulfhemoglobin, showed an absorption in the red region of the spectrum. The characteristic band of this pigment, around  $\lambda 620 \text{ m}\mu$  and adjacent to that of methemoglobin, was not altered by cyanide, although that of methemoglobin was obliterated. Furthermore, comparisons with known mixtures and calculations based upon absorption coefficients for sulfhemoglobin, methemoglobin, and hemoglobin did not correspond with the observed spectrophotometric measurements.<sup>2</sup>

At this time (1940) Fairley's paper appeared describing a new pigment (3) with practically identical properties named methemalbumin (1937, 1938) (4). The pigment, which did not occur in washed, laked erythrocytes nor in urine, was readily formed by incubating laked human erythrocytes with human plasma at  $37^\circ \text{C}$ . for several days. Our observations were in accord with these.

Inasmuch as the sera in cases of acute hemolytic anemia contained hemoglobin, methemoglobin, and apparently methemalbumin (1), it was necessary to obtain absorption coefficients for this new pigment in order to determine its proportion in these sera. Fairley's procedure was, therefore, repeated and the resulting solutions (after centrifuging off the heavy precipitate to be described after further study) were analyzed with the Hardy recording spectrophotometer (5).

To establish the identity of the new pigment, two reagents were used: 5 per cent neutralized  $\text{NaCN}^3$  and 3 per cent hydrogen peroxide. The cyanide was used first to obliterate the absorption

caused by methemoglobin at  $\lambda 630 \text{ m}\mu$  without changing absorption in this region attributed to methemalbumin. Hydrogen peroxide was next added to obliterate the absorption band of sulfhemoglobin, if present.

In Figure 1 are shown the transmission curves of the new pigment, methemalbumin, together with curves of methemoglobin, sulfhemoglobin, and oxyhemoglobin and the changes that negate the absorption in the region of interest.<sup>4</sup> In the case of methemalbumin after the addition of cyanide, the absorption in the red region is decreased slightly owing to the removal of small contaminating amounts of methemoglobin. The subsequent addition of hydrogen peroxide caused no further change. Table I summarizes these findings which correspond to Fairley's results.

TABLE I  
*Differential reactions of the blood pigments*

Reagent*	Methemoglobin $\lambda 630 \text{ m}\mu$ band	Sulfhemoglobin $\lambda 620 \text{ m}\mu$ band	Methemalbumin $\lambda 620 + \text{m}\mu$ band
Cyanide, 5 per cent. $\text{H}_2\text{O}_2$ , 3 per cent....	obliterated obliterated	unaltered obliterated	unaltered unaltered

\* Fairley also used several other reagents which disperse the band of methemoglobin but not the adjacent band of methemalbumin (3,4). Ammonium sulfide caused confusion by subsequently producing a sulfhemoglobin band at  $620 \text{ m}\mu$ . Stokes' reagent, hydrazine hydrate and sodium fluoride might also be used but the results with cyanide alone are quite convincing. The major problem is differentiation from sulfhemoglobin;  $\text{H}_2\text{O}_2$  is the only reagent known to disperse the sulfhemoglobin band but not the methemalbumin band. This test also excludes verdehemochromogens (8).

## *Determination of absorption coefficients*

In Figure 2 is shown the transmission curve *A* of the supernatant of a plasma hemoglobin solu-

<sup>4</sup> For ease of comparison arbitrary concentrations have been selected. Before spectroscopy, all specimens were diluted about 10 times with M/4 phosphate buffer of pH 7.0 to prevent variations in the absorption by methemoglobin with varying pH (2).

<sup>1</sup> Aided in part by the Dazian Foundation.

<sup>2</sup> Absorption coefficients for hemoglobin and methemoglobin taken from Fox and Cline (7) and for sulfhemoglobin taken from Drabkin (2).

<sup>3</sup> Ammonium sulfide may be used but this frequently causes confusion by subsequently producing sulfhemoglobin.

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# SPECTROPHOTOMETRY OF FAIRLEY'S NEW BLOOD PIGMENT, METHEMALBUMIN

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In the course of the study of acute hemolytic anemia and hemoglobinuria during administration of sulfonamide drugs (1), the patients' sera were found to contain a pigment which, like methemoglobin and sulfhemoglobin, showed an absorption in the red region of the spectrum. The characteristic band of this pigment, around  $\lambda 620 \text{ m}\mu$  and adjacent to that of methemoglobin, was not altered by cyanide, although that of methemoglobin was obliterated. Furthermore, comparisons with known mixtures and calculations based upon absorption coefficients for sulfhemoglobin, methemoglobin, and hemoglobin did not correspond with the observed spectrophotometric measurements.<sup>2</sup>

At this time (1940) Fairley's paper appeared describing a new pigment (3) with practically identical properties named methemalbumin (1937, 1938) (4). The pigment, which did not occur in washed, laked erythrocytes nor in urine, was readily formed by incubating laked human erythrocytes with human plasma at  $37^\circ \text{C}$ . for several days. Our observations were in accord with these.

Inasmuch as the sera in cases of acute hemolytic anemia contained hemoglobin, methemoglobin, and apparently methemalbumin (1), it was necessary to obtain absorption coefficients for this new pigment in order to determine its proportion in these sera. Fairley's procedure was, therefore, repeated and the resulting solutions (after centrifuging off the heavy precipitate to be described after further study) were analyzed with the Hardy recording spectrophotometer (5).

To establish the identity of the new pigment, two reagents were used: 5 per cent neutralized  $\text{NaCN}$ <sup>3</sup> and 3 per cent hydrogen peroxide. The cyanide was used first to obliterate the absorption

caused by methemoglobin at  $\lambda 630 \text{ m}\mu$  without changing absorption in this region attributed to methemalbumin. Hydrogen peroxide was next added to obliterate the absorption band of sulfhemoglobin, if present.

In Figure 1 are shown the transmission curves of the new pigment, methemalbumin, together with curves of methemoglobin, sulfhemoglobin, and oxyhemoglobin and the changes that negate the absorption in the region of interest.<sup>4</sup> In the case of methemalbumin after the addition of cyanide, the absorption in the red region is decreased slightly owing to the removal of small contaminating amounts of methemoglobin. The subsequent addition of hydrogen peroxide caused no further change. Table I summarizes these findings which correspond to Fairley's results.

TABLE I  
*Differential reactions of the blood pigments*

Reagent*	Methemoglobin $\lambda 630 \text{ m}\mu$ band	Sulfhemoglobin $\lambda 620 \text{ m}\mu$ band	Methemalbumin $\lambda 620 + \text{m}\mu$ band
Cyanide, 5 per cent.	obliterated	unaltered	unaltered
$\text{H}_2\text{O}_2$ , 3 per cent. . . .	obliterated	obliterated	unaltered

\* Fairley also used several other reagents which disperse the band of methemoglobin but not the adjacent band of methemalbumin (3,4). Ammonium sulfide caused confusion by subsequently producing a sulfhemoglobin band at  $620 \text{ m}\mu$ . Stokes' reagent, hydrazine hydrate and sodium fluoride might also be used but the results with cyanide alone are quite convincing. The major problem is differentiation from sulfhemoglobin;  $\text{H}_2\text{O}_2$  is the only reagent known to disperse the sulfhemoglobin band but not the methemalbumin band. This test also excludes verdehemochromogens (8).

## *Determination of absorption coefficients*

In Figure 2 is shown the transmission curve *A* of the supernatant of a plasma hemoglobin solu-

<sup>4</sup> For ease of comparison arbitrary concentrations have been selected. Before spectroscopy, all specimens were diluted about 10 times with M/4 phosphate buffer of pH 7.0 to prevent variations in the absorption by methemoglobin with varying pH (2).

<sup>1</sup> Aided in part by the Dazian Foundation.

<sup>2</sup> Absorption coefficients for hemoglobin and methemoglobin taken from Fox and Cline (7) and for sulfhemoglobin taken from Drabkin (2).

<sup>3</sup> Ammonium sulfide may be used but this frequently causes confusion by subsequently producing sulfhemoglobin.



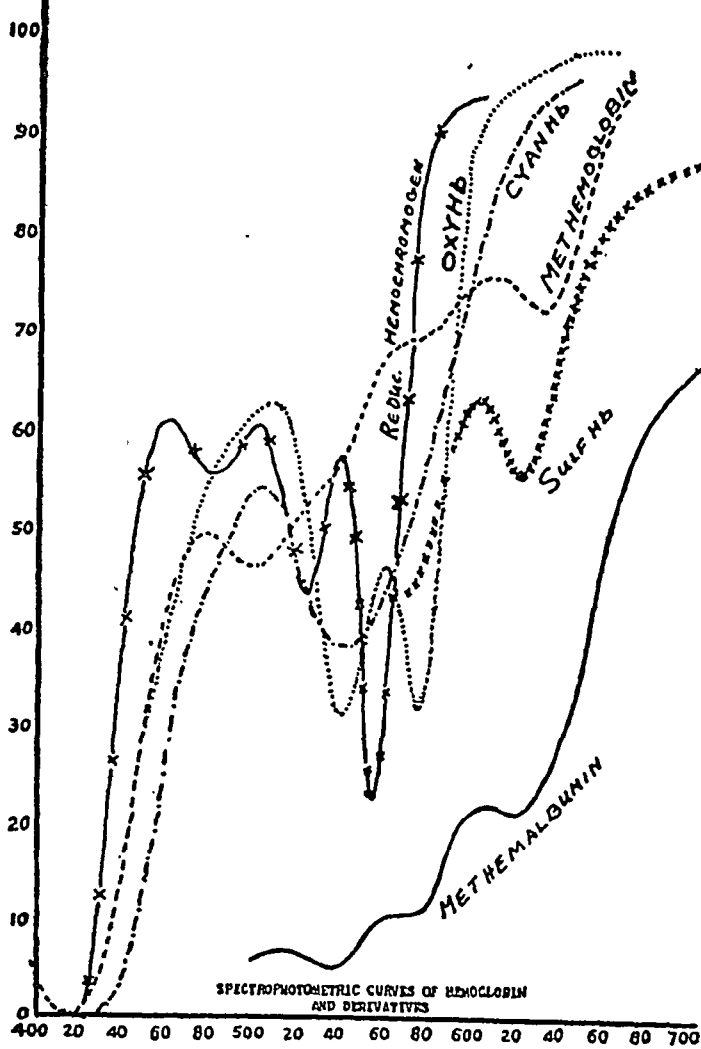


FIG. 1

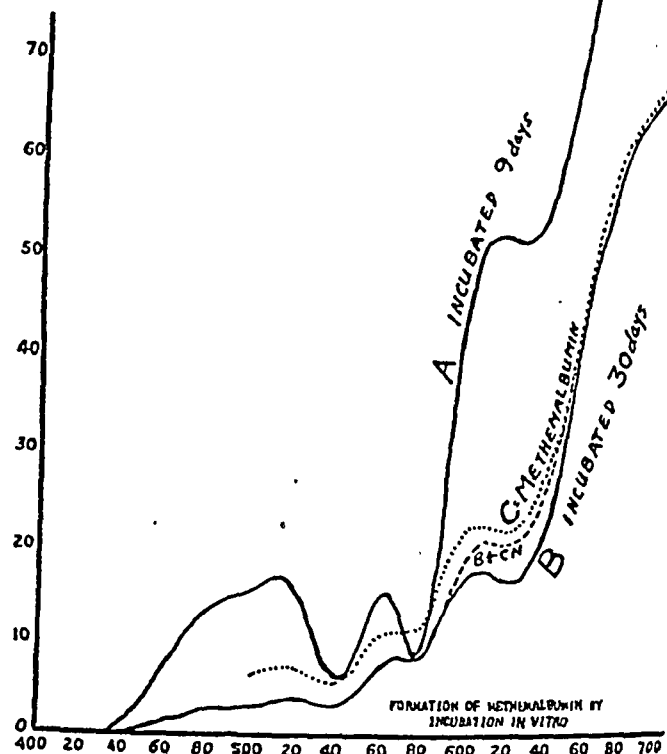


FIG. 2

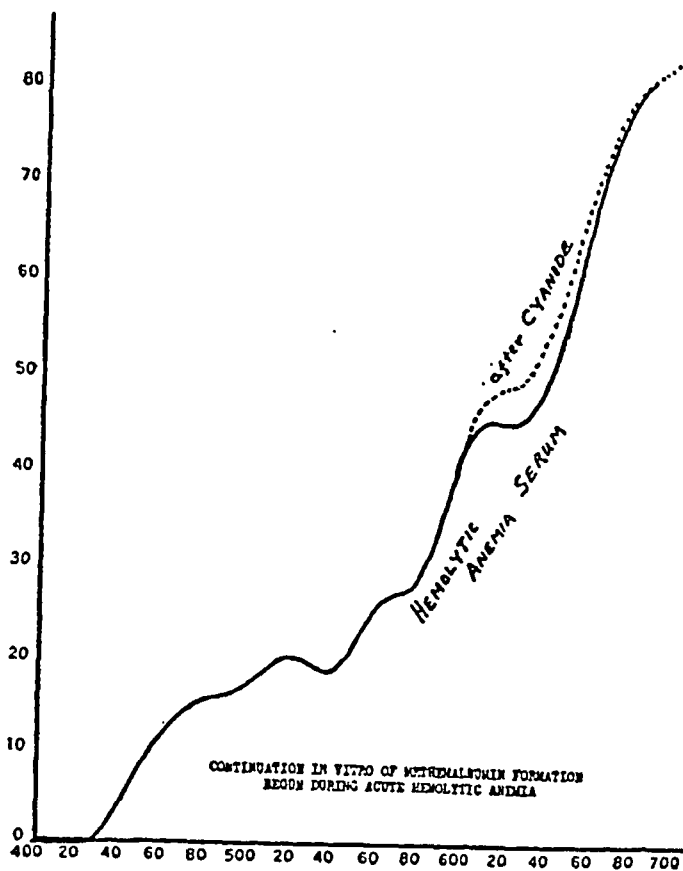


FIG. 3

tion that had not been incubated as long as the solution represented in curve *B*. The hemoglobin bands present in curve *A* had practically disappeared in curve *B*, indicating almost complete disappearance of hemoglobin from the solution. It was then assumed that the curve *B* represented only methemalbumin and methemoglobin. The total pigment was determined by iron analysis with alpha alpha' dipyridyl (6). The methemoglobin, determined independently by the change in optical density at  $\lambda 630\text{ m}\mu$  after the addition of cyanide, was then subtracted from the total to obtain the quantity of methemalbumin present. Since the absorption coefficients of methemoglobin had been determined previously on this instrument (7), it was possible to subtract its absorption from curve *B*, using this formula based on Beer's and Bouguer's laws:

$$\log \frac{1}{T} = kcd$$

$d$  = thickness of cell in cm.

$c$  = concentration in grams per cent

$k$  = absorption coefficient

$T$  = light transmission, per cent.

The calculations are summarized in Table II and the resulting curve *C* of methemalbumin is shown in Figure 2. By the same equation, the absorption coefficients for methemalbumin were computed and are listed in Table II.

TABLE II  
Computation of absorption coefficients of methemalbumin

Wavelength $m\mu$	Experimental $\log 1/T$	Methemoglobin to be subtracted ( $0.0572 \times k \text{ Mhb}$ )	Methem- albumin computed absorption	Methem- albumin absorption coefficients
700	0.177	0.007	0.170	0.72
660	0.323	0.027	0.296	1.25
630	0.751	0.133	0.618	2.61
620	0.788	0.125	0.663	2.79
600	0.770	0.113	0.657	2.77
580	1.059	0.137	0.922	3.89
560	1.112	0.144	0.968	4.08
540	1.456	0.222	1.234	5.2
520	1.414	0.272	1.142	4.83
500	1.502	0.319	1.183	5.0
480	1.55	0.286	1.264	5.34

In order to check the accuracy of the data, the resulting absorption coefficients were used to compute the concentrations of hemoglobin, methemoglobin, and methemalbumin in the solution containing all three represented by curve *A* in Figure 2. The method previously described (7) of solving three simultaneous equations was used. Equations were set up at the peak wavelength of each component  $\lambda 540\text{ m}\mu$ ,  $\lambda 630\text{ m}\mu$ ,  $\lambda 620\text{ m}\mu$ , and solved for the concentration of each component, *e.g.*, at  $\lambda 630\text{ m}\mu$ .

$$\log \frac{1}{T} = 0.133 \times \text{concentration Hb.} + 2.32 \\ \times \text{concentration MHb.} + 2.55 \\ \text{concentration MHA1b.}$$

As a check on the reliability of the entire computation, the values for the concentration of each component were then substituted at 6 other wavelengths and values for the transmission of such a solution were computed. Comparison with the observed values are shown in Table III and serve to establish the relative accuracy of the coefficients of methemalbumin that were obtained.

The absorption coefficients are quite similar to those for methemoglobin and further confirm Fairley's suggestion that "the iron is held in the trivalent state, and is even more resistant to reducing agents than methemoglobin itself" (4).

It was also possible to apply these data to serum obtained from a patient who had suffered acute blood destruction during therapy with sulfonamide drugs (1). This specimen had been measured with the Hardy at the time of the hemolytic crisis.<sup>5</sup> It was then allowed to remain in the refrigerator for 2 months and again measured (Figure 3). The sharp hemoglobin bands originally present had almost entirely disappeared and the band in the red region that resisted both cyanide and hydrogen peroxide had increased markedly; apparently, much more methemalbumin had formed. The methemoglobin was computed by the change in density at  $\lambda 630\text{ m}\mu$  after adding cyanide. The concentrations of hemoglobin and methemalbumin

<sup>5</sup> Case 4 in Bibliography (1).

FIGS. 1, 2 AND 3. TRANSMISSION CURVES WITH THE HARDY RECORDING-SPECTROPHOTOMETER

Ordinates = Per cent of light transmission.

Abscissae = Wavelength of light in  $m\mu$ .

TABLE III  
Check on absorption coefficients of methemalbumin

Wave-length	Hemoglobin ( $0.082 \times k_{\text{Hb}}$ )	Methemoglobin ( $0.04 \times k_{\text{MHb}}$ )	Methemalbumin ( $0.069 \times k_{\text{MHalb.}}$ )	Calculated log 1/T	Experimental log 1/T
$m\mu$	gram per cent	gram per cent	gram per cent		
600	0.046	0.078	0.191	0.316	0.312
580	0.585	0.076	0.27	0.951	0.969
560	0.476	0.101	0.282	0.859	0.824
520	0.338	0.19	0.333	0.861	0.824
500	0.271	0.223	0.345	0.839	0.817
480	0.340	0.200	0.368	0.908	0.88

were computed by two equations<sup>6</sup> at  $\lambda 620 m\mu$  and  $\lambda 540 m\mu$ . Using the concentrations obtained, values for log 1/T at 8 other wavelengths were computed and are compared with the experimental values (Table IV).

TABLE IV  
Formation of methemalbumin at 10° C. after intravascular hemolysis

Wavelength	Experimental log 1/T	Calculated log 1/T
$m\mu$		
700	0.077	0.078
660	0.140	0.142
630	0.328	0.329
620	0.344	0.344
600	0.359	0.342
580	0.538	0.544
560	0.594	0.552
540	0.727	0.727
520	0.699	0.665
500	0.770	0.752
480	0.809	0.724

Values calculated on basis of:

Hemoglobin = 0.01144 gram per cent.

Methemoglobin = 0.0244 gram per cent.

Methemalbumin = 0.1036 gram per cent.

There are some discrepancies between Table III and Table IV; it is recognized that the values for the absorption coefficients in the green are the least reliable and the discrepancies are most

<sup>6</sup> In view of the fact that the method of simultaneous equations is open to criticism when more than two pigments are present, the concentration of methemalbumin was also calculated more directly by using the residual absorption at  $\lambda 620 m\mu$  after cyanide and the coefficient for methemalbumin 2.79 (Table II). After subtracting for the absorption of cyanmethemoglobin, the concentration of methemalbumin found is 0.108 gram per cent. This is 4 per cent more than the result 0.104 gram per cent by the method of simultaneous equations. The difference is presumably due to the fact that, in the residual absorption method, no allowance can be made for the small amount of absorption at this wavelength from the hemoglobin present.

marked in this region. Furthermore, the serum used in Table IV was that of a case of acute hemolytic anemia, and did not give an absolutely clear solution for study.

#### SUMMARY AND CONCLUSIONS

Fairley's new blood pigment methemalbumin was measured in the visible range with the recording spectrophotometer and preliminary values of its absorption coefficients were obtained.

The characteristic absorption curve of this new pigment is compared with that of hemoglobin, methemoglobin, and sulfhemoglobin.

The data obtained are utilized in measuring the formation of methemalbumin, *in vivo* and *in vitro*.

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# THE CAUSE OF DEATH IN EXPERIMENTAL ANURIA<sup>1, 2</sup>

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Winternitz and his associates (1) observed electrocardiographic changes in dogs rendered anuric by bilateral occlusion of renal arteries. These changes were so similar to those which had been previously reported by the present authors in experimental potassium poisoning (2, 3) that a common origin was suspected. A systematic experimental study of the association between anuria and potassium poisoning was therefore undertaken and forms the subject of the present report.

## REVIEW OF LITERATURE

In 1822 Gaspard (4) and Ségalas (5) observed sudden death following the intravenous injection of urine; Voit (6) in 1868 first suggested that potassium might be the poisonous element. Astaschewsky (7) and Feltz and Ritter (8), followed a few years later by Beck (9) and Herringham (10), clearly demonstrated that the toxicity of urine samples varied with their potassium content, although certain other investigators continued to emphasize the importance of other toxic substances in urine (11 to 16). Both Beck and Herringham carefully pointed out that their experiments proved only that urine is toxic, not that potassium is responsible for the symptoms or for the fatal outcome of "uremia."

The suggestion that potassium might accumulate within the body and might be responsible for various "toxic" manifestations of renal disease is not new. Accurate determination of potassium concentration in serum was, however, necessary to establish this hypothesis, and technically reliable methods were unavailable until recently. All older estimations, and many of those published as recently as fifteen or twenty years ago (8, 17, 18), must therefore be accepted with reservations. More recent studies indicate that, although potassium is not regularly increased in the serum of patients with renal disease, even in the presence of severe azotemia (19) and in "uremia," sporadic elevations may be found (19 to 25). On the other hand, increase in the concentration of potassium in serum has been observed with some regularity in experi-

mental animals with ligated ureters (21, 26, 27, 28) and with bilateral renal infarction (1).

## Rationale of present experiments

There is nothing to indicate that the irregular increases in potassium of serum in anuria mentioned above were of sufficient magnitude to cause death, or even to produce lesser evidences of toxicity. Electrocardiographic changes only have been reported (1, 28), without other signs of cardiovascular insufficiency. Substances other than potassium, many of them fatally toxic when injected individually in large quantities, also accumulate during anuria (23). In the absence of any evidence other than its mere presence in serum in increased concentration, it is quite arbitrary to select one or another particular substance and assign to it a primary rôle in causing death.

To know if potassium is responsible for the death of anuric animals, it is essential to follow the electrocardiogram and the serum potassium concentration until the actual moment of death. The course may then be compared with that following intravenous injection of potassium salts in otherwise normal animals (2). A reasonable basis for the conclusion that potassium itself is the cause of death would be provided if the anuric animals should: (1) develop the same sequence of electrocardiographic changes with rising serum potassium as do normal animals (Figure 1); (2) survive until a concentration of serum potassium is attained at which normal animals die; (3) invariably die when such a concentration is reached; and (4) develop the same terminal electrocardiographic changes and die in the same way as do the controls. This conclusion would be strengthened if death occurred at the same concentration of serum potassium in ordinary anuric dogs and in those receiving added potassium, since the two groups would have in common only the single factor of the serum potassium concentration. Conversely, if these conditions should not be ful-

<sup>1</sup> Aided by grants from the Fluid Research Fund of Yale University, the Ella Sachs Plotz Foundation, and from the Council on Therapeutics of the American Medical Association.

<sup>2</sup> Preliminary report presented before the American Physiological Society, April 1941 (Am. J. Physiol., 1941, 133, 331).

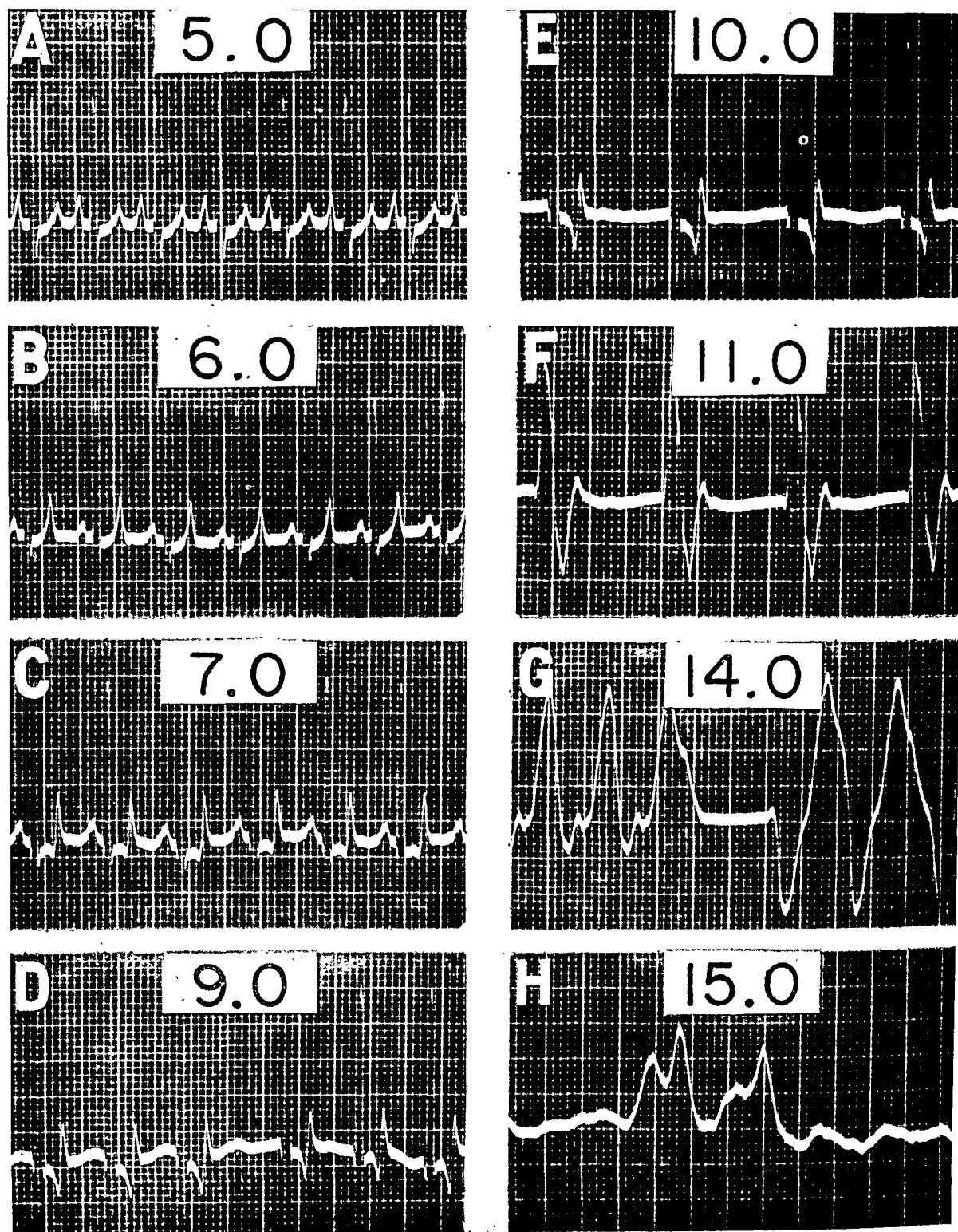


FIG. 1. NORMAL ANIMAL RECEIVING ISOTONIC KCl INTRAVENOUSLY AT A UNIFORM RATE UNTIL DEATH

The figures on each electrocardiogram indicate the usual concentrations of serum potassium associated with the changes it shows.

(A) Control, Lead II.

(B) T waves higher, beginning drop of S-T segment.

(C) Further increase in height of T waves and depression of S-T segment. The P waves are still intact.

(D) Disappearance of P waves. T waves diphasic.

(E) P waves remain absent, T waves diphasic. Beginning widening of QRS complexes.

(F) Marked QRS widening (intraventricular block).

(G), (H) Terminal disorganization of the entire complex.

filled, potassium accumulation is probably not the cause of death in anuria.

Since the validity of this method of attack rests in part on the specificity of the sequence of electrocardiographic changes induced by potassium, they will be described briefly. They are in the sequence in which they appear: (1) progressive heightening of the T wave, which often becomes diphasic, together with a drop in S-T segment, (2) disappearance of the P wave, (3) widening of the QRS complex, indicating intraventricular block, and (4) cardiac arrest.

This sequence is shown in Figure 1, together with the concentration of serum potassium associated with each change. Certain other changes, such as P-R dissociation, sometimes occur. The T wave is characteristically sharp in outline.

Although no individual change is pathognomonic of potassium effect, the sequence is so invariable and so uniquely characteristic when taken in its entirety that it is practically diagnostic of potassium poisoning.

#### METHODS AND MATERIALS

Three groups of dogs were employed. In the first the ureters were tied, in the second the kidneys were removed, while in the third a solution of mercuric chloride was injected intravenously. Operations were performed with full aseptic precautions under anesthesia induced by intraperitoneal nembutal or sodium amytal. Three to four mgm. of mercuric chloride per kilo of body weight were injected slowly into the femoral vein as a 0.1 per cent solution in isotonic saline. All animals given mercury became completely anuric within twelve hours, and in only one did urine flow later recommence, with eventual recovery. In the majority of the experiments food and water were withheld after anuria became established, since free drinking of water resulted in persistent vomiting and secondary chloride depletion. Animals showing dryness of mucous membranes or other evidences of dehydration received intraperitoneal injections of 500 or 1000 cc. of normal saline.

Electrocardiograms were taken from Lead II in all instances, using a Sanborn "Cardiette." Whole blood was usually employed for chemical determination of nonprotein nitrogen. Serum separated from blood allowed to clot under oil was employed for potassium, chloride and calcium analyses. Samples were taken from the femoral vein or, if the animal was moribund, directly from the heart. Nonprotein nitrogen was determined by a micro Kjeldahl technique (29); potassium as chloroplatinate by Hald's modification of the method of Shohl and Bennett (30); calcium by permanganate titration of an oxalate precipitate (31); and chloride by the method of Van

Slyke (29). Before operation and at frequent intervals thereafter electrocardiograms and blood samples were taken. An attempt was made to be present at the actual moment of death, in order to obtain the terminal electrocardiogram and blood sample. This was successfully accomplished in about half of the experiments.

Certain observations on patients with advanced renal disease have been included for comparative purposes. All techniques were identical with those employed in experimental animals.

#### RESULTS

##### *(A) Experiments with anuria due to nephrectomy and to ureteral ligation*

The uncomplicated course of events following bilateral ureteral ligation was studied in twelve dogs; that following bilateral nephrectomy in six others. These two separate modes of producing anuria were employed because their pathological results differ (1, 32). Tables I and II, respectively, summarize these groups of experiments. The results in both groups are evidently very similar. Those of a chemical nature are: (1) progressive increase of serum potassium and of blood nonprotein nitrogen throughout the course of each experiment; (2) irregular decrease of serum chloride concentration; and (3) little change in calcium concentration. The electrocardiographic findings are: (1) changes in the T wave demonstrable within twenty-four to forty-eight hours, first appearing at potassium concentrations of 6 to 7 mM. per liter, and persisting in progressively intensified form throughout the course; (2) disappearance of P waves, usually after seventy to 100 hours, with serum potassium concentration of 9 to 11 mM. per liter; and, finally, (3) progressive widening of QRS complexes, followed within a few minutes, or at most within a few hours, by cardiac arrest and death. The widening of the QRS complex occurred only after the P wave had disappeared, and was associated with concentrations of serum potassium ranging from 12 to 20 mM. per liter. These electrocardiographic changes are illustrated in Figure 2, taken from a typical experiment following nephrectomy, and in Figure 3, taken from one with ureteral ligation. The changes are virtually indistinguishable from those in Figure 1 produced in normal animals by the intravenous injection of potassium. Furthermore, the concentrations of

potassium associated with each of these various changes are approximately the same as those observed in the animals with experimental potassium poisoning (2, 3).

No correlation could be found between the deficits of chloride sometimes observed (Table I, Experiments 1, 2, 3, 4, 8, and 13) and electrocardiographic changes. The large deficits in the first three experiments were due to the free administration of water by mouth, which induced severe vomiting. In the remainder of the experiments water was withheld and occasional injections of normal saline were given intraperitoneally, thus largely preventing chloride deficits. Since serum calcium was but little affected, it may be excluded as a disturbing factor.

Potassium chloride solutions in varying amounts were injected intraperitoneally into four dogs with

ligated ureters and into five nephrectomized dogs immediately following the establishment of anuria. The results are summarized in Table III. The electrocardiographic sequence in a typical experiment is presented in Figure 4. This sequence is indistinguishable in character and progress from that of a normal animal receiving potassium continuously (Figure 1), and from that in anuric dogs without intraperitoneal injection of potassium (Figures 2, 3). The chemical changes are also qualitatively similar to those in anuric dogs receiving no potassium.

There are, however, significant quantitative differences between the group with uncomplicated anuria and the group receiving potassium. In Figure 5 are plotted the concentrations of potassium in serum at death in normal animals poisoned with potassium, in those with uncomplicated

TABLE I  
*Ureters ligated. Experiments in which no potassium was determined*

Number	Time after ligation	Death	Blood NPN	Serum			Electrocardiographic findings			Remarks
				K	Cl	Ca	T wave changes	P waves disappear	QRS widening	
1	hours		mgm. per 100 cc.	mM. per liter	mM. per liter	mgm. per 100 cc.				Water given. Much vomiting. Saline given once. Death not observed.
	0		41	6.0	108.6		0	0	0	
	44		174	8.0	81.6		+	0	0	
	72		216	10.0	78.2		+	+	0	
	72-84	+								
2	0		29	5.9		9.1	0	0	0	Water given. Vomiting. Infusion. Death not observed. (Blood obtained after death.)
	24						+	0	0	
	49		174	6.4	87.6	9.0	+	0	0	
	71						+	+	±	
	77-78	+		19.9						
	78		259	19.9	47.0	9.9				
3	0		29	6.6	105.8	11.0	0	0	0	Water given. Vomiting. Infusion. Death not observed.
	36		95	8.3	97.2		+	0	0	
	48		124	9.8	94.8		+	0	0	
	74			10.6	87.6	10.8	+	0	0	
	92		177	8.8	86.0		+	+	0	
	107		194	9.9	89.6	10.2	+	+	0	
	115						+	0	0	
	125			10.5	89.2	10.7	+	+	±	
	128		221	11.2	90.4	9.8	+	±	±	
	132-139	+								
4	0		34	6.3			0	0	0	Death not observed.
	49						+	0	0	
	72		222	9.9	89.6		+	+	0	
	79-87	+								
5	0		25	5.9	109.4	11.3	0	0	0	One convulsion. Gradual decline to death.
	53						+	0	0	
	70		169	9.7	109.4	10.4	+	0	0	
	122						+	+	0	
	142	+	341	14.5	104.6		+	+	+	

TABLE 1—Continued

Number	Time after ligation	Death	Blood NPN	Serum			Electrocardiographic findings			Remarks
				K	Cl	Ca	T wave changes	P waves disappear	QRS widening	
6	hours		mgm. per 100 cc.	mM. per liter	mM. per liter	mgm. per 100 cc.				Convulsion.
	0		26	5.9	109.4	9.8	0	0	0	
	53						+	0	0	
	70		204	8.5	113.6	10.2	+	0	0	
	82						+	+	0	
7	84						+	+	+	Gradual decline to death.
	113	+	391	11.9	98.4	6.7	+	+	+	
	0		27	5.1	108.6	10.9	0	0	0	
	70		185	7.7	112.0	11.0	+	0	0	
	126						+	+	+	
8	141	+	375	20.4	105.4	9.7	+	+	+	Convulsion. Bloody diarrhea. Sudden loud cry, arrest of heart, dyspnea, death.
	0		29	4.6	107.8		0	0	0	
	66		220	11.8	86.8		+	0	0	
	79						+	+	+	
	107						+	+	+	
10	111	+	378	12.3			+	+	+	Death not observed.
	0		24	4.6	104.6		0	0	0	
	73		161	11.8	93.6		+	+	0	
	91-93	+								
11	0		41	4.7	109.4		0	0	0	Death not observed. Blood 1 hour post-mortem.
	24			6.4	107.8		+	0	0	
	49			9.5	105.4	11.1	+	+	0	
	65		379	9.2	114.0		+	0	0	
	68						+	+	0	
12	72						+	+	+	Cardiac failure, dyspnea, finally respiration stopped.
	82-83						+	+	+	
	83	+		14.3	103.8	11.7	+	+	+	
						9.7				
13	0		30	4.4	101.4	10.4	0	0	0	Gradual decline to death.
	49						+	0	0	
	92		226	7.6	100.6	11.6	+	0	0	
	103						+	+	+	
	106	+	354	12.4	103.6	11.2	+	+	+	
	0		26	3.5	106.0		0	0	0	Gradual decline to death.
	49						+	0	0	
	72		166	8.5	104.6		+	0	0	
	113						+	+	+	
	152	+	346	17.1	66.4		+	+	+	

anuria, and in those with anuria receiving potassium. The range of terminal potassium concentration is the same in the three groups. In Figure 6 the rise in nonprotein nitrogen is plotted against the increase in serum potassium. There is no difference between the results with nephrectomized dogs (open circles) and those with dogs in which ureters were ligated (open squares). However, animals in which added potassium had been injected (solid circles and squares) had a lower blood nonprotein nitrogen at death than did the uninjected animals having equivalent increases in serum potassium. This fact, taken in conjunc-

tion with the demonstration in Figure 5 that animals in both series died with the same concentration of potassium, indicates that death is correlated with a certain degree of elevation of serum potassium rather than with a certain increase in the blood nonprotein nitrogen. This conclusion is further borne out in Figure 7, in which the times of survival of the animals in the various groups are plotted. There is no difference in the duration of life of nephrectomized animals and those with ligated ureters. There is, however, a striking decrease in the survival time of those receiving potassium. This might have been predicted fr



TABLE II  
*Nephrectomized dogs, no K injected*

Num- ber	Time	Death	Blood NPN	Serum			Electrocardiographic findings			Remarks
				K	Cl	Ca	T wave changes	P waves disap- pear	QRS widen- ing	
N 1	<i>hours</i>		<i>mgm. per 100 cc.</i>	<i>mM. per liter</i>	<i>mM. per liter</i>	<i>mgm. per 100 cc.</i>				Sudden groan, followed by death.
	0		39	5.6	107.0		0	0	0	
	65		192	8.9	98.4		+	0	0	
	72						+	+	0	
	147						+	+	+	
	148						+	+	+	Sudden death.
	152	+	411	15.3			+	+	+	
N 2	0		27	5.4	104.6		0	0	0	
	65		176	8.5	88.2		+	0	0	
	72						+	+	0	
	97						+	+	+	
	117						+	+	+	Sudden death, preceded by dyspnea.
	125	+	394	18.0			+	+	+	
N 3	0		50	4.8	107.8		0	0	0	
	65		211	9.6	90.8		+	0	0	
	72						+	+	0	
	95						+	+	+	
	100						+	+	+	Sudden death.
	116	+	378	16.0			+	+	+	
N 4	0		47	4.3	101.4		0	0	0	
	48			6.8	85.6	9.4	+	0	0	
	72		292	11.8	69.8		+	+	+	
	76	+	363	12.3	74.4	9.7	+	+	+	
										Gradual cessation of respira- tion and heart. (Coarse ventricular fibrillation.)
N 6	0		44	4.8	98.2		0	0	0	
	48						+	0	0	
	72		211	8.3	88.4	11.9	+	0	0	
	104						+	+	0	
	125						+	+	+	
	126	+	322	13.4	94.4	9.5	+	+	+	

the demonstration (Figure 5) that both groups have the same concentration of potassium at death.

(B) *Experiments with anuria due to the injection of mercuric chloride*

Eleven experiments in which mercuric chloride solution was injected are summarized in Table IV. Blood nonprotein nitrogen and serum potassium rose regularly and progressively, just as they did in the animals with surgically induced anuria. As in the other types of anuria, changes in the T wave of the type ascribed to potassium appeared early and regularly in these animals with the anuria of mercury poisoning. However, the group with mercury poisoning differs fundamentally from the nephrectomized group and from the group with ligated ureters in at least three respects: (1) In three of the five instances in which terminal serum potassium concentrations were determined, a con-

centration less than 10 mM. per liter was found. This is less than the minimal lethal concentration in normal animals. Of the five in which it was not determined, four died within twenty-four to thirty-six hours after the establishment of anuria. These four therefore probably had serum potassium concentrations well below 10 mM. per liter, if the same rate of increase of potassium concentration be assumed in these as in the other experiments. Thus, only two, or possibly three, out of eleven dogs could have had terminal concentrations of potassium compatible with death due to potassium poisoning. (2) P waves were retained in all but two experiments. In experiment Hg 8 P waves were retained and the QRS complex was not widened even at the very end. Electrocardiograms from one of the two exceptional instances (Hg 9) in which P waves did disappear are presented in Figure 8. (3) The survival time was

on the average much shorter than in dogs rendered anuric by surgical means (Figure 7). This is true in spite of a considerable variation in the time of survival, due in part to the different doses of mercury used in different experiments.

### (C) Clinical observations

The results of chemical and electrocardiographic studies in five patients with advanced chronic pyelonephritis and glomerulonephritis are summarized in Table V. In all instances the serum

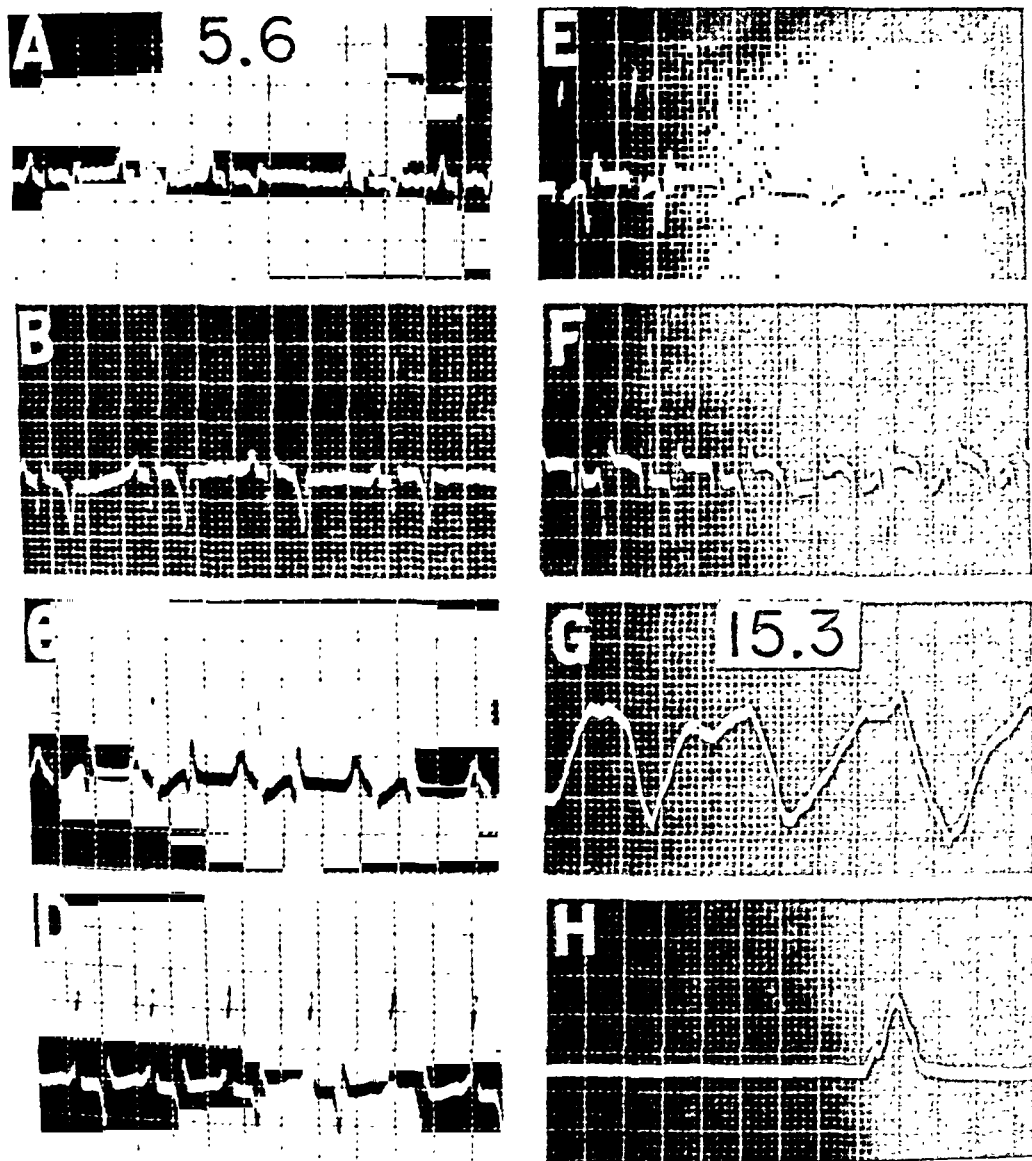


FIG. 2. NEPHRECTOMIZED DOG. NO KCl INJECTED

- (A) Control, Lead II.
- (B) 96 hours after nephrectomy. P waves still present. T waves inverted.
- (C) 108 hours. P waves present, S-T segment depressed, T waves diphasic.
- (D), (E), (F) 120 to 124 hours. P waves have disappeared, S-T segment depressed. QRS high but of normal width. Animal walking about.
- (G) 125 hours. Complete disorganization of QRS complexes. Serum potassium 15.3 mM. per liter.
- (H) 1 minute later. Death.

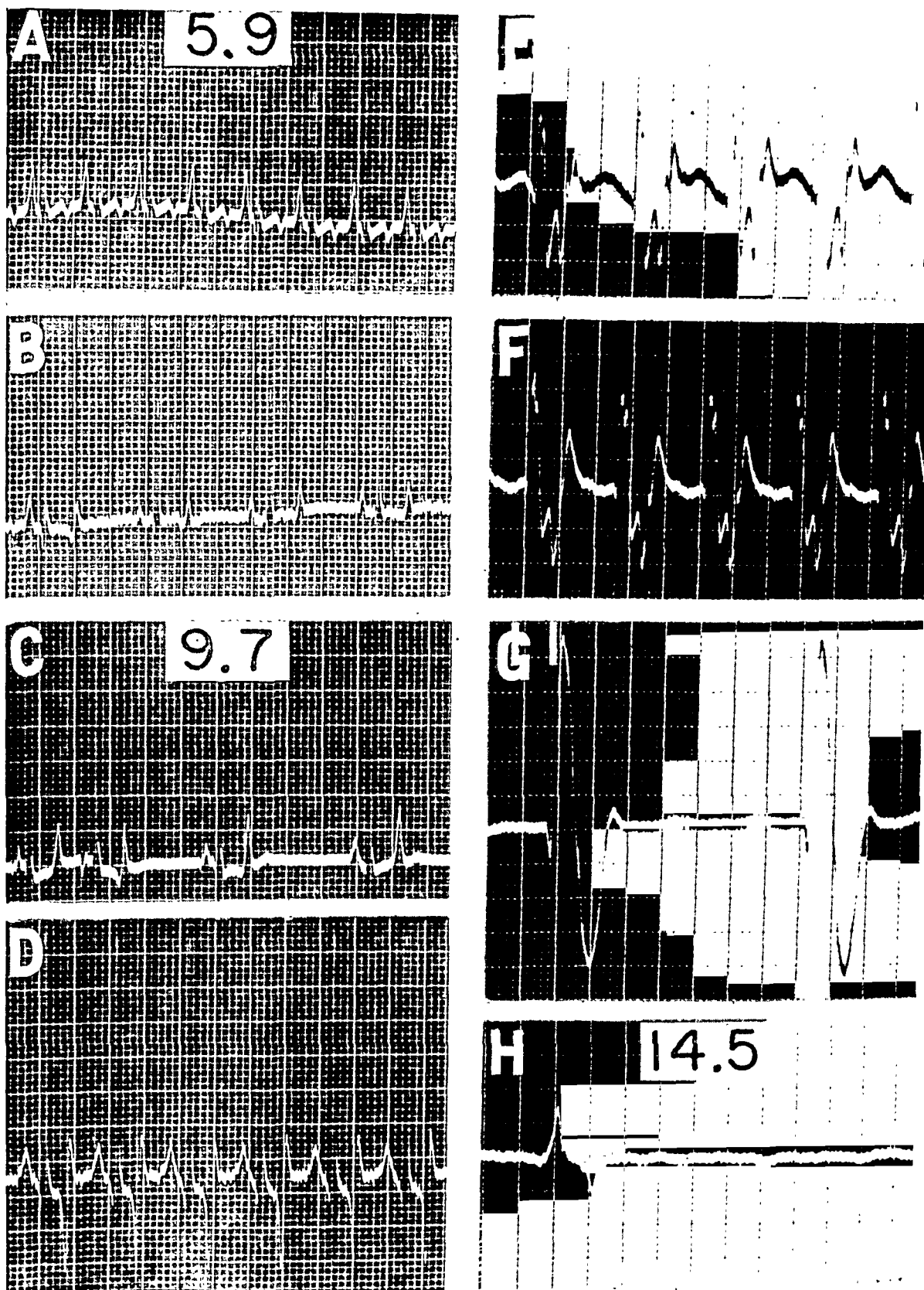


FIG. 3. URETERS TIED. NO KCl INJECTED

- (A) Control, Lead II.  
 (B) 48 hours after operation. T waves slightly diphasic.  
 (C) 72 hours. P waves present. S-T depression. Serum potassium 9.7 mM. per liter.  
 (D), (E), (F) Series taken at intervals during 12 hours preceding death. Loss of P waves, widening of QRS complex, further T wave changes.  
 (G), (H) Terminal records, showing complete absence of P, and extreme intraventricular block. Serum potassium 14.5 mM. per liter.

TABLE III

*Ureters tied or nephrectomized, KCl injected intravenously immediately following ligation*

Number	Time after ligation	Amount of K injected isotonic	Death	Blood NPN	Serum		Electrocardiographic findings			Remarks
					K	Cl	T wave changes	P waves disappear	QRS widening	
14	hours 0 18 24 30	cc. per kilo 22.7		mgm. per 100 cc. 27	mM. per liter 4.8 9.8	mM. per liter 107.2 106.4	0 + +	0 + +	0 0 0	Died very suddenly having been walking about apparently normally 20 minutes before death.
15	0 21 40 53 55	less than 22.7*		32	5.1 6.8	104.4 106.4	0 + + +	0 0 + +	0 0 + +	Sudden death preceded by dyspnea.
17	0 18 29 49 54	20.0		29	4.1 8.7	103.2 102.4	0 + + +	0 0 + +	0 0 0 +	Sudden death following 10 minutes of dyspnea.
20	0 18 29 40 42	20.0		29	5.4 8.8	108.0 101.6	0 + + +	0 0 + +	0 0 0 +	Sudden death.
N 7	0 19 74 106 108	15.0		31* 80*	5.0 7.4		0 + + +	0 0 + +	0 0 0 0	? Sudden death. Not actually observed.
N 8	0 19 31 50 52	15.0		33* 88*	5.1 9.3		0 + + +	0 0 + +	0 0 0 +	Sudden dyspnea, then death.
N 9	0 18 73 94 96	15.0		36* 88*	5.6		0 + + +	0 0 + +	0 0 + +	Exact moment of death not observed.
N 10	0 10 67 68	15.0		29 48	5.2 5.4		0 + +	0 0 +	0 0 0	Death not observed.
N 11	0 9 29 61 74 80	15.0		30 60	4.9 6.1		0 0 + + +	0 0 + + +	0 0 0 + +	Death not exactly observed.

\* Unknown amount of solution lost.

\* Serum determination.

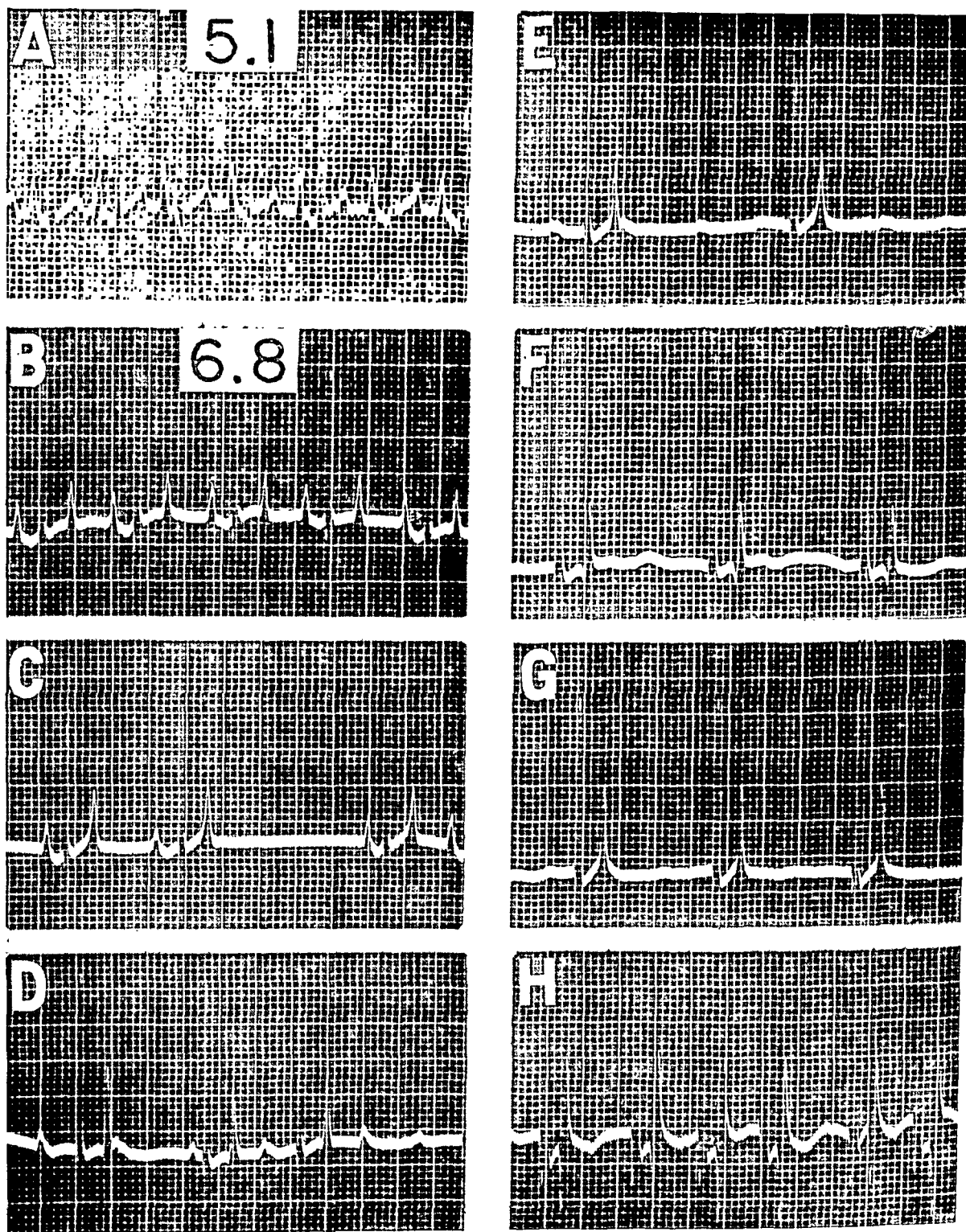


FIG. 4. URETERS TIED, KCl INJECTED INTRAPERITONEALLY IMMEDIATELY FOLLOWING LIGATION  
 (A) Control, Lead II.  
 (B) 21 hours after ligation. Little change. P waves present. Serum potassium 6.8 mM. per liter.  
 (C), (D), (E) 21 to 38 hours. Progressive diminution and widening of P waves. Some PR block. T waves become progressively higher.  
 (F) 40 hours. P wave has disappeared.  
 (G) 44 hours. Progressive increase in height of T; P remains absent. QRS complex intact.  
 (H) 55 hours. Beginning QRS widening. Death occurred 2 hours later. Serum potassium 13.3 mM. per liter.

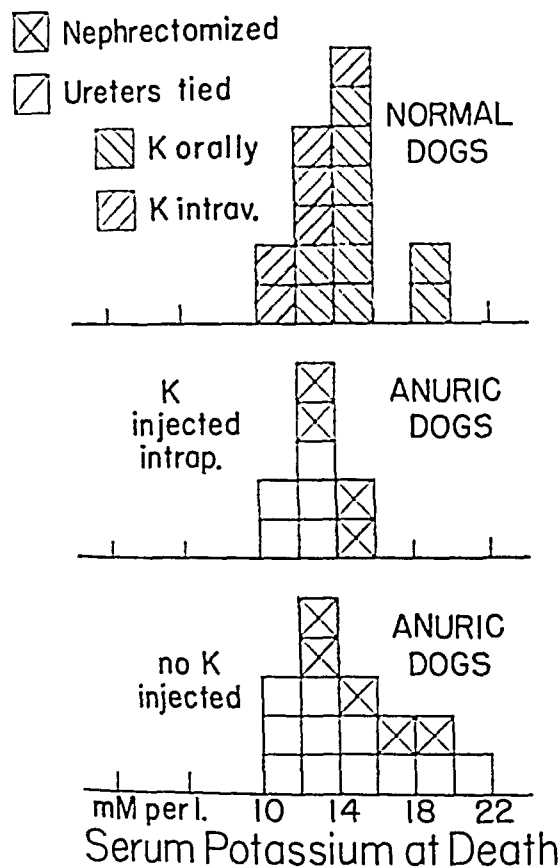


FIG. 5. CONCENTRATION OF POTASSIUM AT DEATH

Each square represents one experiment. There is evidently no difference between the concentration in the anuric experiments and in the controls. There is also no difference between the concentrations in the anuric experiments with potassium administration and those without potassium injection.

potassium concentration was but little elevated, although the blood nonprotein nitrogen was extremely high. The electrocardiograms showed no changes characteristic of potassium poisoning. This is true in spite of the fact that several of the electrocardiograms were taken just before death. One of these is shown in Figure 9, in which the last records were taken ten minutes before respiration ceased; P waves and QRS complexes are intact. The associated potassium concentration in serum was 6.9 mM. per liter.

#### DISCUSSION

Potassium regularly accumulates in the serum of anuric animals even when food is withheld and

no potassium salts are injected. Since its rise roughly parallels that of the blood nonprotein nitrogen (Figure 6), it is reasonable to assume that it results from the same cause, *i.e.*, from the breakdown of body cells.<sup>3</sup> Such an explanation, of course, implies inability on the part of the body to excrete the potassium by any channel other than the kidney or to store any considerable amount. The variable rate of increase of the serum potassium, together with the fact that it may occasionally even drop for a time (Table I, experiment 3), indicates that the tissues may have some ability to store potassium. The generally progressive character of the increase, however, indicates that this capacity is strictly limited.

Winkler and Smith (33) observed that potassium injected into normal dogs distributes itself through a volume considerably larger than that of the extracellular fluid. Potassium was therefore under these circumstances able to enter cells and at least temporarily to be stored there. The present experiments with injections of potassium into anuric dogs indicate a similar large apparent volume of distribution. In Table VI apparent volumes of distribution of added potassium are calculated for all the experiments in which potassium was injected. (It is assumed that, after a period of twelve hours, absorption from the peritoneum is complete, yet potassium release from tissue breakdown is negligible.) Since the extracellular fluid corresponds only to about 25 per cent of the body weight, considerable potassium must have entered cells in these experiments. Especially striking are experiments N 9, N 10, and N 11, in all of which only a small fraction of the injected potassium could be accounted for in the extracellular fluid the next morning. In spite of this demonstrated capacity of potassium to leave the extracellular fluid to enter cells, the concentration in serum gradually rose in subsequent days until death ensued. Storage of potassium under these circumstances is therefore definitely limited in

<sup>3</sup> The ratio of the increase in serum concentration of nonprotein nitrogen to that of potassium in these experiments is about one gram per liter of the former for every 2 or 3 mM. per liter of the latter. Assuming their volumes of distribution to be similar, these concentrations are proportional to total amounts. The ratio is consistent with the hypothesis that all the increase in potassium and nitrogen is derived from tissue breakdown.

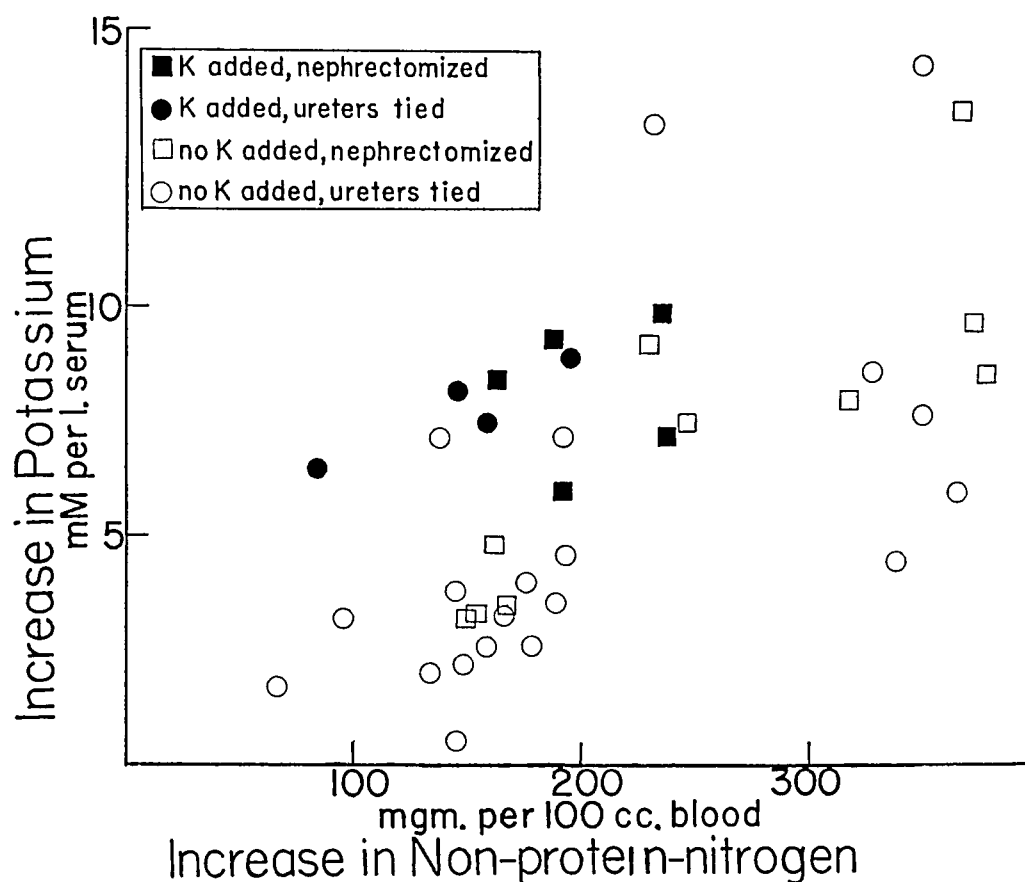


FIG. 6. INCREASE IN BLOOD NONPROTEIN NITROGEN ( $\Delta$  NPN) AND SIMULTANEOUS INCREASE IN SERUM POTASSIUM ( $\Delta$  K)

All blood samples in which these two determinations were carried out are included. The association is evident, but the points scatter rather widely.

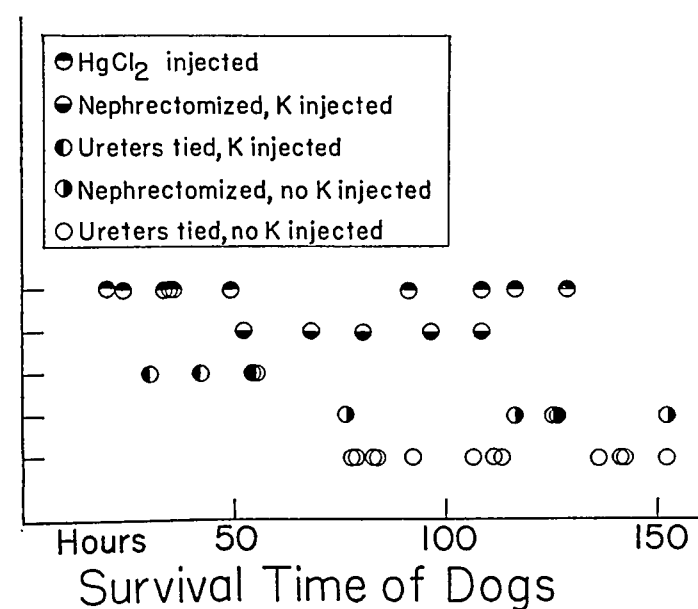


FIG. 7. SURVIVAL TIME OF THE ANIMALS IN DIFFERENT GROUPS OF EXPERIMENTS

Each circle represents one experiment. The time is estimated from the onset of anuria. The animals receiving potassium evidently die much sooner on the average than those receiving none. There is a wide dispersion of the experiments with mercury poisoning, but many of the animals died in a shorter period than did any of those with simple surgical anuria.

amount. No clue is available from these experiments concerning the form in which the potassium is stored in cells, nor is it clear whether it enters all cells or only certain specialized ones.

The survival of animals with surgically induced anuria until potassium attains a level in serum sufficient to produce death is only presumptive evidence that potassium is the effective lethal agent. More conclusive is the fact that serial electrocardiograms invariably demonstrate a sequence identical with that produced by experimental potassium poisoning. That the electrocardiographic sequence in anuria is truly due to potassium is confirmed in a negative way by the studies of human subjects with uremia, in which the serum potassium was not elevated in spite of the high concentration of nonprotein nitrogen and of other products in the blood. In such patients no electrocardiographic changes of the sort observed in experimental anuria could be found. It was observed in the animals that the terminal cardiac slowing and arrest occurred quite suddenly, although blood pressure and cardiac output had presumably been well maintained. This mode of

TABLE IV  
*Mercury poisoning*

Number	Time after injection	Amount HgCl <sub>2</sub> given	Death	Blood NPN	Serum K	Electrocardiographic findings			Remarks
						T wave change	P wave out	QRS widening	
Hg 2	hours 0 24 30 30-38	mgm. per kgm. 4.0	+	mgm. per 100 cc. 41	mM. per liter 5.7	0 + +	0 0 0	0 0 0	Not observed.
Hg 3	0 24 73 89 90 90-91	4.0	+	24   352	5.7  7.8 9.0 10.2	0 + + + +	0 0 0 + +	0 0 0 0 +	Not observed.
Hg 4	0 24 30 30-38	4.0	+	42	5.6	0 + +	0 0 0	0 0 0	Not observed.
Hg 5	0 24 48 48-49	4.0	+	33  166	5.1  6.0	0 + +	0 0 0	0 0 0	Not observed.
Hg 7	0 21 80 104 104-112	3.5	+	28	7.1	0 + + +	0 0 0 0	0 0 0 0	Not observed.
Hg 8	0 20	3.5	+	125 184	6.9 9.2	0 +	0 0	0 0	Gradual disappearance of respirations; sudden ventricular flutter and then coarse fibrillation.
Hg 9	0 22 80 92 94 116	3.5	+	33  317  370	6.1  9.8  13.2	0 + + + +	0 0 0 + +	0 0 0 0 +	Short period of dyspnea, then sudden death.
Hg 10	0 20 22-26	3.5	+	34		0 +	0 0	0 0	Not observed.
Hg 11	0 20 78 121+	3.0	none	34	6.2  10.5	0 + + 0	0 0 0 0	0 0 0 0	Recovered.
Hg 12	0 20 79 128	3.0	+	30  387	5.6  6.4 9.3	0 + + +	0 0 0 0	0 0 0 0	Died suddenly.
Hg 13	0 20 30 30-40	3.0	+	24		0 + +	0 0 0	0 0 0	Not observed.



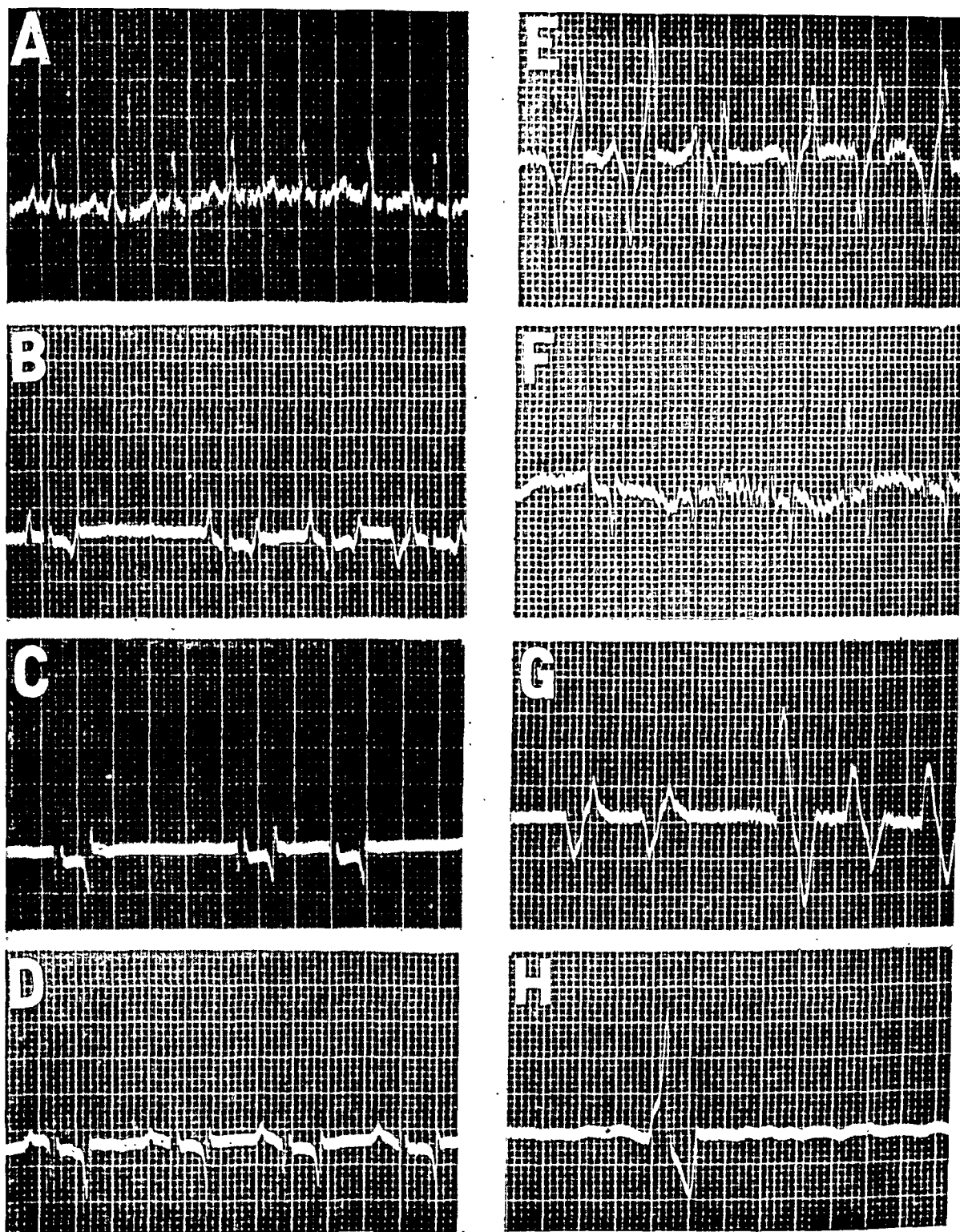


FIG. 8. MERCURY POISONING

- (A) Control, Lead II.  
 (B) 30 hours after injection of mercury. T wave diphasic.  
 (C) 92 hours. P waves have disappeared, drop in S-T segment.  
 (D) 102 hours. P waves return temporarily.  
 (E), (F), (G) 116 hours. Animal dyspneic. P waves have again disappeared, T waves very large, QRS complexes widening and disorganizing.  
 (H) A few minutes later, at moment of cessation of respiration. Serum potassium 13.2 mM. per liter.

TABLE V  
Summary of clinical observations

Number	Diagnosis	Date	Potassium of serum	Blood NPN	Remarks
80838*	Chronic pyelonephritis	September 12, 1940	mM. per liter 6.9	mgm. per cent 208	Died 10 minutes later.
A 3408	Chronic pyelonephritis	August 27, 1940 morning evening	6.7 6.5	256 225	Anuric for preceding 2 or 3 days. Died 2 hours after second blood sample.
B 1929	Chronic glomerulonephritis	April 18, 1940 July 13, 1940	5.0 5.8	122 253	Died 12 hours later.
A 90560	Chronic glomerulonephritis	August 28, 1940	3.8	190	Convulsions at this time. Died 14 days later.
B 5156	Chronic glomerulonephritis	September 29, 1940	5.4	118	Still living.

\* See Figure 9 for electrocardiograms in this case.

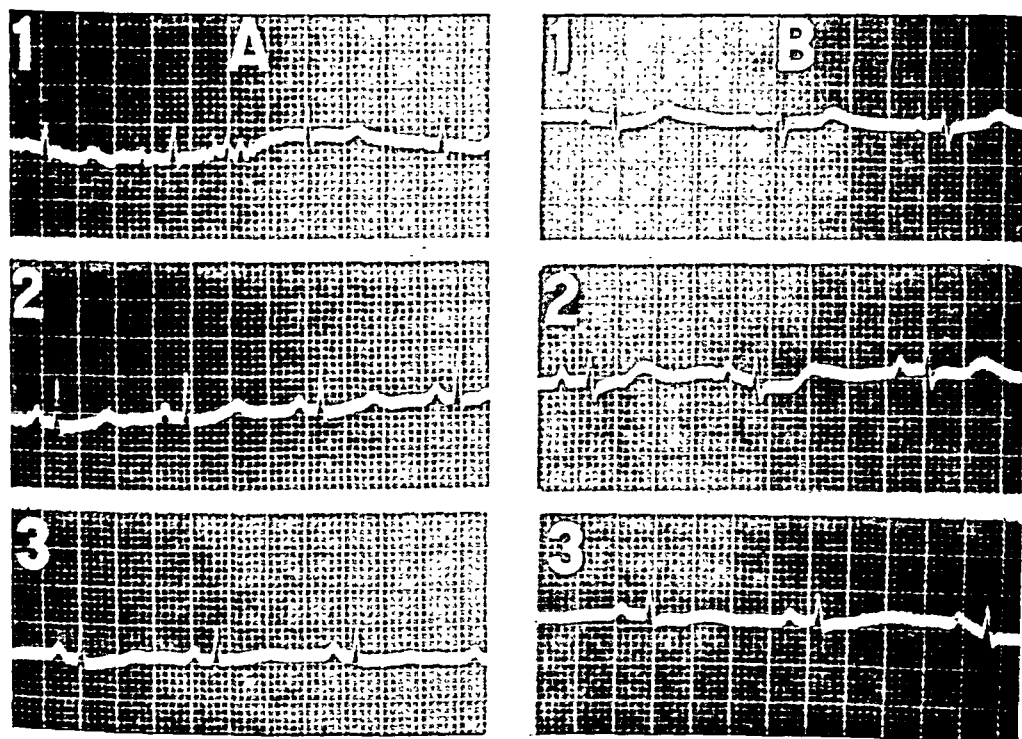


FIG. 9. ELECTROCARDIOGRAMS FROM A YOUNG PATIENT WITH LONG-STANDING CHRONIC PYELONEPHRITIS, JUST BEFORE DEATH

Numbers refer to the respective leads. (A) 24 hours before death; (B) 10 minutes before death. The serum potassium was 6.9 mM. per liter and the blood NPN 208 mgm. per cent just before death. Note that in (B) the P waves are still present and the T waves show no characteristic changes.

TABLE VI  
*Apparent volumes of distribution*

Experiment	Increase in potassium concentration (A)	Amount of potassium injected (B)	Apparent volume of distribution of potassium $\frac{(B)}{(A)} \times 100$
	<i>mM. per liter serum water*</i>	<i>mM. per liter body weight</i>	<i>per cent of body weight†</i>
U 14	5.4	3.50	65
U 17	5.0	3.08	62
U 20	3.7	3.08	83
N 7	2.6	2.31	89
N 8	4.5	2.31	51
N 9	1.0±	2.31	very large
N 10	0.2	2.31	very large
N 11	1.3	2.31	178

\* Assuming serum water = 93 per cent of serum.

† Assuming 1 liter = 1 kilo.

death is entirely consistent with that due to potassium poisoning (2). The alternative possibility is that some other substance retained in the body to the same extent as potassium might be the true cause of death, the rise in potassium with its correlated electrocardiographic changes being a non-essential accompaniment. This seems, however, adequately ruled out by the experiments in which added potassium was introduced into the system after anuria was established. Death occurred in the same way with the same electrocardiographic sequence and at the same range of concentration of potassium as in the control series, but at a much earlier time and with distinctly less elevation of blood nonprotein nitrogen. Other workers (34, 35) have reported that diets high in potassium decrease the survival time of anuric rats. Were death really dependent on the accumulation of some toxic substance other than potassium, the time of death would not be accelerated in this way and the terminal concentration of potassium would be expected to be higher than usual.

Altogether, the evidence seems conclusive that death in surgical anuria is caused by cardiac poisoning due to the accumulation of excessive potassium in the body fluids. The difficulty of generalizing concerning other forms of anuria is evident from the experiments with mercury poisoning. Here anuria occurred and potassium of serum rose, as in the other experiments. However, before the potassium reached levels sufficient to

cause death and before electrocardiograms showed more than the earlier signs of potassium effect, the animals usually died. The cause of death is uncertain, but it clearly was not potassium poisoning. It may be conjectured that if the animals with mercury poisoning had lived as long as did the dogs rendered anuric by surgical means, they too might have died of potassium poisoning. This may have been the case in the two instances in which P waves disappeared and the terminal serum potassium concentration was greater than 10 mM. per liter. Since most of them did not live so long, potassium poisoning in mercurial anuria must be considered as a limiting cause of death, which is ordinarily not operative, as some other factor is usually responsible for death before sufficient potassium has accumulated.

The clinical studies demonstrate clearly that potassium poisoning is not the usual cause of death in terminal nephritis, since neither elevation of concentration of potassium nor cardiac damage detectable by the electrocardiogram was sufficient to cause death. This is undoubtedly correlated with the fact that even in very advanced nephritis anuria does not usually occur until a few hours or at most a day or so before death. In another study of patients with nephritis Winkler, Hoff and Smith (3) found that amounts of potassium sufficient to keep the serum concentration nearly normal were excreted even when clearances were much depressed. In other words, so long as the nephritic subject excretes any urine at all he eliminates appreciable amounts of potassium, so that the accumulations observed in experimental anuria are not apt to occur. The possibility may be considered that potassium may be the cause of death in those occasional nephritic patients with persistent extreme oliguria or anuria, but such cases have not been encountered in the course of the present study.<sup>4</sup> The high concentrations of potassium in serum occasionally reported in nephritis probably originate from extreme oliguria and some of the recorded values are certainly high enough to be in themselves dangerous to the heart (23).

<sup>4</sup> Dr. Daniel Darrow has observed one child with acute glomerulonephritis and extreme oliguria for several days. His serum potassium shortly before death was 9.3 mM. per liter. No electrocardiograms were obtained.

## CONCLUSIONS

1. The concentration of potassium in the blood serum increased regularly and progressively in animals rendered completely anuric. This increase results from the breakdown of the animal's own tissues, together with the restricted ability of the organism to store potassium.

2. Serum potassium continues to increase in dogs rendered anuric by ureteral ligation or nephrectomy until cardiac arrest due to potassium poisoning occurs. Thus potassium poisoning is the usual effective cause of death in animals so treated.

3. Dogs rendered anuric by mercuric chloride injections usually die of some unknown cause before the concentration of potassium rises sufficiently to cause cardiac arrest.

4. Elevation of serum potassium with consequent cardiac arrest is not the usual cause of death in patients with chronic nephritis and terminal uremia.

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# THE THIAMIN CONTENT OF HUMAN BLOOD AND URINE AS DETERMINED BY THE FERMENTATION METHOD<sup>1</sup>

By ROBERT GOODHART WITH THE TECHNICAL ASSISTANCE OF THEODORA NITZBERG

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In 1937, Schultz, Atkin and Frey (1) published the first of a series of articles on the stimulating effect of thiamin upon the rate of alcoholic fermentation by yeast. Subsequently they described the experimental conditions under which this activity could be taken advantage of to measure the thiamin content of biological materials. The stimulating effect of thiamin was found to be complicated by an additional stimulation of the pyrimidine half of the molecule (2-methyl-5-hydroxymethyl-6-aminopyrimidine). They stated that similar or related compounds were inactive, although they did not mention the substances tested (2).

As 2-methyl-5-hydroxymethyl-6-aminopyrimidine has not been shown to occur in nature, except as a component of, or a degradation product of, thiamin, its presence probably does not seriously invalidate the method as a means for estimating blood and urine thiamin values in the human subject.

Schultz, Atkin and Frey (3, 4) recently described a method for the determination of what they termed the "true" thiamin content of urine. In this method they took advantage of the fact that the oxidation of thiamin to thiochrome renders it biologically inactive. They measured the total fermentative power of a sample of urine and subtracted from this the fermentative power of an oxidized aliquot portion. The resulting value represented free pyrimidine in the urine. We attempted this procedure but have discarded it because of inaccuracies resulting largely from incomplete oxidation.

These workers also tested the effect of added adenylic acid in their system and found it to be inactive (5). Nicotinic acid was found to have

a stimulating effect in the presence of thiamin (5). This effect was of a very small order and was maximum with a concentration of 1 mgm. per cent of the acid. Nicotinamide was found to have an activity of the same order as nicotinic acid. Schultz, Atkin and Frey therefore modified their technique to include this concentration of nicotinic acid in their stock solution (5). However, as the concentration of nicotinic acid in blood is only about 0.36 mgm. per cent, and as the fermentation method, when applied to the estimation of thiamin in blood, requires the use of only 0.1 cc. of this substance, the amount of nicotinic acid in the blood could not be an important source of error. As the concentration of nicotinic acid in urine is of the same order, the above statement also holds true for urine.

We have used the fermentation method for the study of the blood and urine concentrations of thiamin in normal human subjects and in subjects suffering from a variety of diseases.

## EXPERIMENTAL

The method used for the determination of thiamin in both blood and urine was that described by Atkin, Schultz and Frey as the "Ultramicrodetermination" method (6). We made only two modifications: The first was in the preparation of the thiamin stock solution, which was made up so that 1 cc. contained 2.5 mgm. of thiamin in 30 per cent acid alcohol. The stock solution was stored in the ice box. The amount required for each experiment was obtained by evaporating a sufficient quantity of the stock solution to dryness, and then on the day of the experiment, making up to the correct dilution with distilled water. Second, the yeast suspension was freshly prepared for each experiment.

The bloods used for the estimation of their thiamin content were obtained from fasting subjects. Two mgm. of potassium oxalate per cc. of blood were used to prevent clotting. We have not been able to demonstrate any inhibitory or adjuvant effect of this concentration of potassium oxalate upon the fermentation of glucose by bakers' yeast, either in the presence or the absence of crystalline thiamin.

Urinary estimations were done on 24-hour urine sam-

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ples, collected in bottles containing 10 cc. of 5N. Hydrochloric acid. Urine collected this way was found to remain stable for several days, if stored in the ice box. When simultaneous blood and urine estimations were performed, the blood was drawn at the end of the 24-hour urine collection period. For the estimation of normal blood and urine values, samples were obtained from interns, research workers, and patients on the wards of the Psychiatric Division of Bellevue Hospital who presented no clinical manifestations of somatic or mental disease other than psychoneurosis.

Each experimental run included, in addition to the unknowns and the yeast blanks, a determination of the fermentative effect of at least one known amount of thiamin. All determinations were made in duplicate.

As the sensitivity of the method permitted the use of only very small amounts of blood and urine, greater accuracy was obtained by diluting fairly large quantities of the unknown to an extent that allowed the pipetting of 1 cc. of the final dilution directly into the fermentation vessel. For example, when 0.1 cc. of blood was to be used, 5 cc. of blood were diluted with 0.5 cc. of the gelatin solution and distilled water sufficient to bring the total volume up to 50 cc. One cc. of this solution was then pipetted into the fermentation vessel.

When tests for the recovery of thiamin were run, the thiamin was added to the blood or urine before the dilutions were made.

In Figure 1 are charted typical curves of response of 5 mgm. of yeast<sup>2</sup> to thiamin. The response of yeast to thiamin varies not only with each lot of yeast, but also with each suspension made. Because of this, it is necessary to run at least one point on the curve with each experiment. All the curves fall off with increasing rapidity as the amounts of thiamin increase. It is necessary, therefore, to use amounts of the unknown which contain no more thiamin than the amount used for the portion of the curve determined with that particular experimental run. As the curve is steepest with amounts of thiamin below 0.01 microgram, greater sensitivity and accuracy are obtained when amounts of the unknown giving values less than this are used. We have found that, with normal and deficient bloods, 0.1 cc. is the optimum amount. The corresponding amount for urines was found to be 0.01 cc. When unusually large amounts of thiamin have been administered to the subject before the collection of the blood and urine, smaller amounts of these materials should be used for the determinations.

<sup>2</sup> Supplied by Dr. Charles N. Frey, Fleischmann Laboratories, New York.

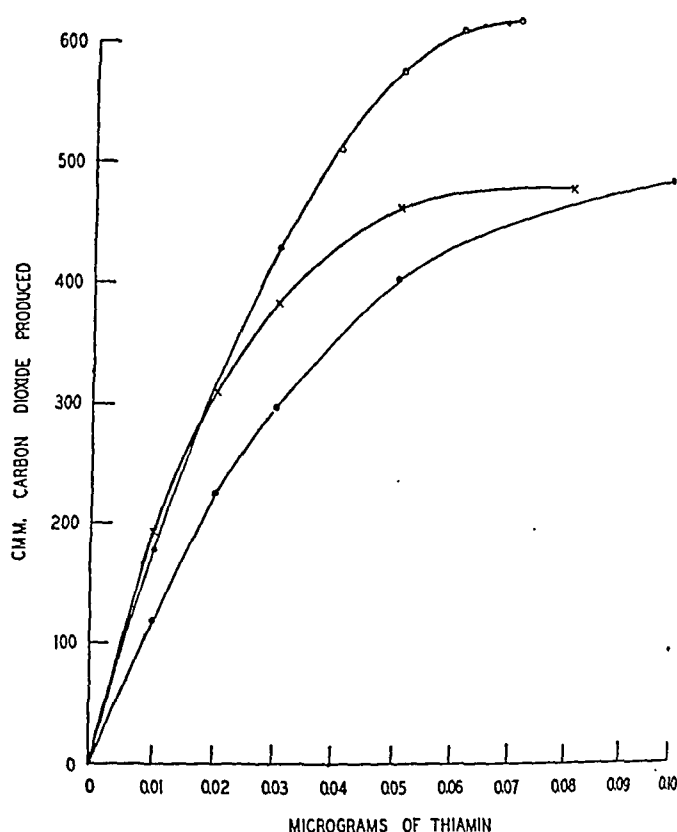


FIG. 1. THE ADJUVANT EFFECT OF THIAMIN UPON CARBON DIOXIDE PRODUCED BY GLUCOSE FERMENTATION WITH 5 MGm. PORTIONS OF BAKERS' YEAST

Atkin, Schultz and Frey prepared their protein samples for analysis by heating them at 100° C. for 30 minutes, after they had been made acid to congo red (6). We tried the effect upon the gas production of heating the bloods, without acidification, in a boiling water bath for varying time intervals (Table I). Increasing the time of heating proved to decrease the amount of gas evolved with

TABLE I  
Effect of duration of heating upon apparent thiamin content of 0.1 cc. of blood

Experiment Number	Minutes heated	Micro-liters CO <sub>2</sub> produced*	Micro-grams of thiamin	Experiment Number	Minutes heated	Micro-liters CO <sub>2</sub> produced*	Micro-grams of thiamin
1	3	166±2	0.0113	4	3	80±2	0.0067
	15	93±1	0.0063		6	70±4	0.0058
					9	70±0	0.0058
2	3	124±2	0.0074	5	3	93±4	0.0052
	9	115±3	0.0069		15	88±3	0.0049
	12	119±2	0.0071				
	15	103±2	0.0061				
3	3	159±4	0.0072	6	3	80±1	0.0065
	12	66±2	0.0053		12	66±2	0.0053
	6	142±3	0.0064		15	67±3	0.0054

\* Average of duplicate determinations ± deviations from average.

TABLE II

*Relation of duration of heating to recovery of thiamin from 0.1 cc. of blood and 0.01 cc. of urine*

Minutes heated	Sample	Micrograms in sample	Micrograms added	Micrograms recovered	Per cent of added thiamin recovered
3	Blood	0.00367	0.01	0.01567	120
3	Blood	0.00367	0.02	0.02907	127
4.5	Blood	0.0067	0.01	0.0169	102
4.5	Blood	0.0067	0.02	0.0257	95
6	Blood	0.00865	0.01	0.01735	87
6	Blood	0.00865	0.02	0.02585	86
3	Urine	0.00855	0.01	0.01925	107
3	Urine	0.00855	0.02	0.03235	119
4.5	Urine	0.0033	0.01	0.0144	102
4.5	Urine	0.0033	0.02	0.0253	105

1 cc. of blood. Studies on the recovery of thiamin, added to bloods and urines, were therefore performed after heating the samples for different time intervals (Table II). It was found that the best recoveries were obtained in the case of both blood and urine when 4.5 minutes was allowed for the period of heating.

It would seem from these experiments that there exists in blood a heat labile factor which stimulates the fermentative action of thiamin. Prolonged heating of non-acidified blood tends to destroy the biological activity of thiamin, or at least in some way to remove it from the reaction.

As a result of these experiments, we adopted the procedure of heating the bloods and urines, after diluting and pipetting into the fermentation vessels, for 4.5 minutes in a boiling water bath.

## RESULTS

Eighty-six determinations of the thiamin content were made on bloods drawn from 45 presumably normal subjects. The average value found for these bloods was 5.39 micrograms per cent, with a range from 3.1 to 9.2 micrograms. The distribution of these values is shown in Figure 2.

In 1939, Goodhart and Sinclair published values for the cocarboxylase content of normal human bloods (7). They found a range from 4.5 to 12.0 micrograms per cent with a mean of 7.0. These findings were essentially confirmed by Goodhart (8) in a later work with the same method. Ex-

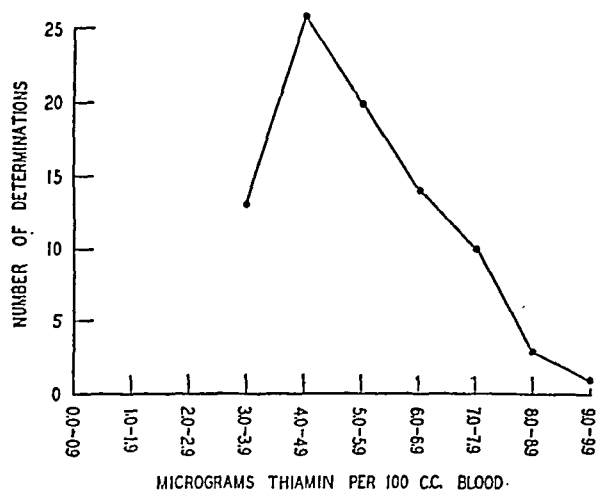


FIG. 2. DISTRIBUTION OF NORMAL BLOOD THIAMIN LEVELS—86 DETERMINATIONS ON 45 SUBJECTS

pressed as thiamin, this would be equivalent to a mean blood content of 4.76 with a range from 3.06 to 8.16 micrograms per cent. As the free thiamin content of human bloods is quite small, these figures agree reasonably well with those given above for the total thiamin content of normal human bloods.

The 24-hour urinary excretion of thiamin was also determined in 11 normal subjects (42 determinations). The range was found to be from 240 to 1327 micrograms, with an average of 596 micrograms for the 24-hour urinary excretion. The distribution of these values is shown in Figure 3.

Considerably higher values are obtained for the apparent thiamin content of urine when the fermentation method is used than is found by most other procedures. This is due to the large urinary content of 2-methyl-5-hydroxy-methyl-6-aminopyrimidine, a substance which also stimulates the fermentation of glucose by yeast. From the work of Schultz, Atkin and Frey (4), it would appear

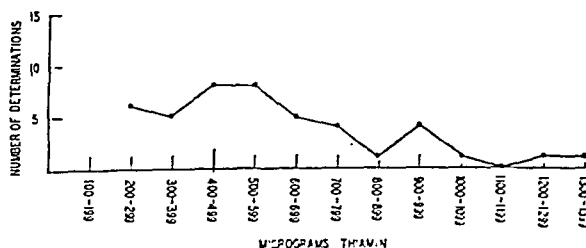


FIG. 3. DISTRIBUTION OF 24-HOUR URINARY EXCRETION VALUES OF 11 NORMAL SUBJECTS—42 DETERMINATIONS



TABLE III

*Relation of 24-hour urinary excretion of thiamin to fasting blood level and to dietary intake in normal subjects*

Subject	Dietary intake	Vitamin calorie ratio	Blood thiamin	Urinary excretion	Intake excreted
	<i>micro-grams</i>		<i>micro-grams per cent</i>	<i>micro-grams</i>	<i>per cent</i>
L	1211	2.35	6.2	506	41.8
	1455	2.47	4.1	606	41.6
	1045	2.03	7.2	820	78.4
	1073	2.04	5.0	682	63.6
S	898	2.28		250	27.8
	1328	2.51	6.8	384	28.9
	1283	2.56	4.3	362	28.3
	1222	2.06	4.3	795	65.9
	1397	2.83	5.4	539	38.6
	1409	2.44	5.0	502	35.6
	1493	2.79	4.9	798	53.4
B	1063	2.21	6.2	929	86.8
	1045	2.01	5.7	721	69.0
	1455	2.47	5.9	1016	69.8

that this pyrimidine may account for anywhere from 23 to 75 per cent of the "total thiamin" content of normal human urines, as estimated by the fermentation method. This means that the absolute figures obtained for urinary thiamin by the fermentation method, as used in our work, are not analogous to those obtained by other methods.

Schultz, *et al.* found the average daily urinary output of 5 normal adults, 32 determinations, to be  $497.7 \pm 47$  micrograms (9). We found a much greater variation in the urinary excretion of thiamin, not only in different subjects but in the same individual from day to day. The explanation for this may rest, in part, in the fact that our subjects were mostly hospital patients, representing the lowest income group of the community. Their previous levels of tissue saturation with the vitamin may therefore have been both low and variable. However, the range of values found in 19 determinations on 4 interns was identical with that of the whole group. The individual interns showed differences between their highest and lowest daily urinary excretions of 410, 65, 113 and 337 micrograms, respectively. As our subjects were maintained with unrestricted diets and unrestricted activity, the finding of large variations in the urinary excretion of thiamin was not unexpected.

The diets of 3 of our subjects were carefully noted during the periods of urine collection and

the vitamin intake was computed. On reference to Table III it will be seen that, in spite of their very moderate intake of thiamin, they excreted a relatively large percentage in the urine, none excreting less than 27.8 per cent. In these subjects, whose dietary intake ranged from 898 to 1493 micrograms of thiamin daily, no correlation could be found between the urinary excretion, the intake, the vitamin/calorie ratio of the diet, or the fasting blood level of thiamin. It would appear that marked variations in the daily urinary excretion of thiamin may occur in normal subjects maintained with fairly constant daily intakes. This must be considered when attempting to evaluate the significance of 24-hour urinary excretions.

In Figure 4 are tabulated the results of the blood thiamin determinations on 106 patients from the wards of the Psychiatric Division of Bellevue Hospital. A solid line is drawn across the graph

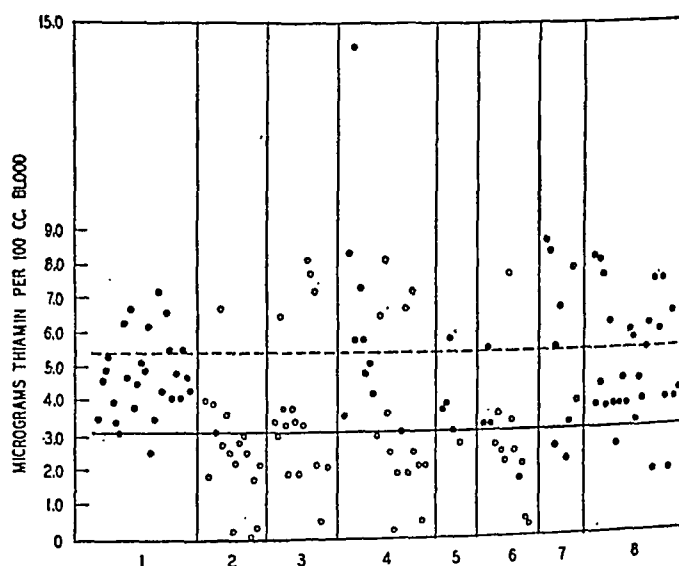


FIG. 4. BLOOD THIAMIN VALUES OF 106 SUBJECTS FROM THE WARDS OF THE PSYCHIATRIC DIVISION OF BELLEVUE HOSPITAL

● represents results on subjects without polyneuropathy.  
○ represents results on subjects with polyneuropathy.

The column numbers (figures on horizontal scale) denote the following conditions:

- (1) "Uncomplicated alcoholics."
- (2) Acute "alcoholic" polyneuropathy.
- (3) Chronic "alcoholic" polyneuropathy.
- (4) The fatty and cirrhotic livers of the inebriate.
- (5) "Alcoholic" pellagra.
- (6) "Alcoholic" encephalopathies.
- (7) Korsakoff psychosis.
- (8) Non-alcoholic patients with a variety of medical and psychiatric disorders.

at the lowest observed normal value. A broken line is drawn at the level of the average value found for our group of "normal" subjects.

The 27 "uncomplicated alcoholics" plotted in column 1 were admitted to the hospital with diagnoses as diverse as lobar pneumonia and acute alcoholism. All were alike in showing no clinical evidences of any vitamin deficiency. All but one had blood thiamin levels within normal limits, albeit the average of their values was considerably below that of the normal group. The one subject who had a definitely subnormal blood thiamin content was admitted to the hospital suffering from acute alcoholic intoxication and hypoglycemic shock. He was treated with glucose infusions and the blood thiamin determination was made on the following day, at which time he was asymptomatic. The possible relationship of this succession of events to his low blood thiamin content is obvious.

The results on subjects with acute "alcoholic" polyneuropathy are listed in column 2; those with chronic neuropathy, in column 3. Calf muscle tenderness and solar dysesthesia were taken as the necessary criteria for the diagnosis of an acute neuropathy. No attention was paid to the history of the duration of the disease as such histories, from alcohol addicts, are worthless. Twelve of the 17 subjects with acute neuropathy had blood thiamin levels definitely below the lowest observed normal value. Four of the remaining 5 subjects had blood values which were definitely low normal. In contradistinction to these results, 10 of the 16 subjects who presented the signs of chronic neuropathy alone had blood thiamin values within the normal range. As there is no reason to believe that these two conditions are not different stages of the same disease, it would seem that in the second instance we are dealing, in many cases, with the residuum of an earlier, acute process which is characteristically associated with low blood thiamin values. It is not meant to imply that thiamin deficiency is the only possible etiological agent for all the polyneuropathies which may occur in the alcohol addict. The evidence presented here neither confirms nor denies the validity of this supposition.

Low blood thiamin values were found in subjects with fatty and cirrhotic livers, "alcoholic"

encephalopathic states, alcoholic pellagrins, and Korsakoff psychosis only when peripheral neuropathy was also present. This finding correlates very well with our clinical impressions acquired from therapeutic trials of thiamin hydrochloride in these different disease states. We have noted, for example, a complete lack of improvement in fatty and cirrhotic livers, when treated with large doses of thiamin hydrochloride. At the same time, we have observed that the peripheral neuropathy, when present, improved as rapidly in these subjects, when treated with thiamin, as it did in the absence of clinical evidence of liver disease. Again, the polyneuropathy of the pellagrin has been shown to respond to treatment with crystalline thiamin. Of 3 subjects with Wernicke's encephalopathy, all had low blood thiamin values and all had peripheral neuropathy. This is in accord with the findings of Jolliffe, *et al.* (10) and we believe constitutes confirmatory evidence that this condition is closely associated with thiamin deficiency. Bowman, *et al.* (11), were unable to obtain any substantial evidence, from therapeutic trials with the vitamin, that thiamin deficiency played a rôle in the production of Korsakoff's psychosis.

The findings on 28 non-alcoholic patients presenting a variety of medical and psychiatric disorders are recorded in column 8, Figure 4. This group comprised 9 subjects with general and cerebral arteriosclerosis, 5 with tuberculosis, 2 with hypertensive encephalopathy, 2 diabetics, 1 of whom had severe acute and chronic peripheral neuropathy, 4 schizophrenics, and 1 case each of psychoneurosis, manic-depressive psychosis, pernicious anemia, rheumatic arthritis, benign spinal cord tumor and lympho-epithelioma of the nasopharynx. Three definitely low blood values were found, one in a subject with general and cerebral arteriosclerosis, one in a case of miliary tuberculosis and the third in the subject with lympho-epithelioma. The distribution of the blood thiamin values in the remainder of this group was not significantly different from that of the normal control group.

The high incidence of peripheral neuritis (18 out of 22 cases) found among those subjects who had blood thiamin values below 3.1 micrograms per 100 cc. suggests to us the probability that such low blood values may be indicative of the exist-

nance of definite thiamin deficiency states in the human subject.

#### SUMMARY

We elected to use the fermentation method for the studies reported in this paper because it appeared to us, at the time, that this method offered certain very definite advantages over all other available methods for the determination of thiamin in body fluids. First, the procedure of the fermentation method is simple and rapid and, although the initial cost of equipment is rather large, the subsequent expense is minimal. Second, the method promised to be much more applicable to routine estimations on hospital and clinic patients than cocarboxylase determinations since, in the former, live yeast is used, and the enzyme system is considerably more stable. Third, the fermentation method is immensely more sensitive than the "diazo method" used by Melnick and Field (12). Fourth, the thiochrome method in our hands has proved rather unsatisfactory, an experience which we know to have been shared by many other investigators; witness the large number of modifications of the thiochrome method. As a result of our work, we have been convinced of the genuineness of these advantages.

There exists in blood a heat labile factor which stimulates the fermentative action of thiamin. Prolonged heating of non-acidified blood at 100° C. tends to destroy the biological activity of its thiamin. Optimal results are obtained with the fermentation method when the blood or urine is heated at 100° C. for 4.5 minutes.

An average value of 5.39 micrograms per 100 cc., with a range from 3.1 to 9.2, was obtained for 86 determinations on bloods drawn from 45 normal subjects.

Forty-two determinations of the 24-hour urinary excretion of thiamin, on 11 normals, gave an average of 596 micrograms, with a range from 240 to 1327. The urinary excretion of thiamin in normal subjects was found to depend upon factors

other than the total thiamin intake and the vitamin/calorie ratio of the diet.

A very definite association was found to exist between the acute peripheral neuropathy of the alcohol addict and low blood thiamin values. The incidence of peripheral neuropathy among subjects with low blood values was so high as to make it seem likely that further work will prove the finding of a blood thiamin value below 3.0 micrograms per cent, by the fermentation method, to be a definite indication of a thiamin deficiency state.

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# NOTE ON THE INTERPRETATION OF CLEARANCE METHODS IN THE DISEASED KIDNEY

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In interpreting the results obtained by clearance methods in the diseased kidney, the physiological limitations of these methods must be kept clearly in mind. Since there has been no opportunity to discuss these limitations generally, it has seemed desirable to emphasize certain more important points in this note.

The four methods to be considered here are the inulin clearance ( $C_{IN}$ ), the plasma diodrast clearance ( $C_D$ ), the maximal rate of tubular excretion of diodrast ( $Tm_D$ ) and the maximal rate of tubular reabsorption of glucose ( $Tm_G$ ), all four methods being based on overall measurements made on the two kidneys by the collection of bladder urine (4, 10, 12).

## DEFINITIONS

It will aid in the following discussion if certain terms are given restricted definitions.

A *normal active nephron* designates a patent glomerulus with its tubule—both glomerulus and tubule functioning, by virtue of an adequate supply of blood delivered at an adequate pressure, in the formation of glomerular filtrate and in tubular excretion and reabsorption.

At least by definition, the glomeruli and tubules of normal nephrons may become partly or wholly *inactive* due to ischemia without losing their potentiality for immediate return of function on the restoration of an adequate blood supply.

An *aglomerular tubule* is one in which excretory function and urine formation persist after destruction of the adjoined glomerulus. (There is as yet no functional evidence of the existence of aglomerular tubules in the normal or diseased human kidney; it remains to be demonstrated that the mammalian nephron, which normally reabsorbs water, can acquire the capacity to excrete it, and the excretion of water must be presupposed if the term "aglomerular nephron" is to have functional significance.)

An *impotent nephron* is conceived as one in which the tubule remains anatomically intact and connected with an active glomerulus and a patent collecting duct despite the fact that it has lost its excretory activity, as tested by diodrast. Such a tubule would act as a more or less passive conduit to drain glomerular filtrate into the urine. Whether such impotent nephrons simultaneously lose all other tubular properties, such as the capacity to reabsorb glucose, chloride, water, etc., is not to be answered *a priori*

but only by investigation; but since by definition they are unable to excrete diodrast they do not contribute to  $C_D$  or  $Tm_D$ .

All renal parenchyma which normally lacks excretory function, or which has lost its excretory function, will be designated as *inert tissue*. This would include capsular and connective tissue, impotent nephrons as defined above, injured nephrons which permit the back-diffusion of inulin and other constituents of the tubular urine, nephrons which are obstructed by casts or disconnected from collecting ducts so that urine formation is impossible, and fibrotic glomeruli and tubular fragments generally.

## Total renal function and function in individual nephrons

Recognizing that there are two million-odd nephrons in the two kidneys, it is self-evident that no overall method of examination can directly reveal what is occurring in individual nephrons. The total clearance method cannot reveal whether impairment in any of the above functions is the result of the partial reduction of function in all nephrons or the complete reduction of function in a few. Conversely, constancy of these functions does not imply constancy in all contributing nephrons, since function may be increased in some at a time when it is decreased in others.

The above considerations are most obviously applicable to  $C_{IN}$  and  $C_D$ , which in their physiological nature must be presumed to be highly labile in any one nephron. But to a lesser degree these considerations also apply to  $Tm_D$  and  $Tm_G$ ; although these measurements are based on saturation methods and presumably represent the maximal attainable activity in all available nephrons, nevertheless the availability of a particular nephron depends, in the case of  $Tm_D$ , on perfusion by blood, and in the case of  $Tm_G$ , on glomerular filtration; hence alternation of activity and partial, as compared with complete, cessation of activity in individual nephrons are theoretically possible. There is also the possibility that the functional contribution of indi-

vidual nephrons may be related to "trophic" or other influences. For example, White, Heinbecker and Rolf (16) have recently shown that  $Tm_D$  is decreased in hypophysectomized dogs; hypertrophy of the kidneys occurs in animals receiving large doses of androgens and estrogens (5, 6, 7, 8, 9), and the hypertrophy of the kidney induced by a high protein diet is well known. While it has not yet been shown that either  $Tm_D$  or  $Tm_G$  is increased in these hypertrophic kidneys, a functional change is to be anticipated.

#### *Filtration of water and the inulin clearance*

The term "glomerular filtration" may be used primarily to designate the passage of water across the glomerular membranes. Since a variable quantity of water is reabsorbed by the tubule, it is impossible, either in a single nephron or in the total kidneys, to deduce from the urine flow how much water is filtered from the blood. The rate of filtration of water can only be deduced in either case by means of an appropriate standard of reference, namely, a completely filtrable solute which is itself neither reabsorbed nor excreted by the tubule; and in turn the selection of this standard of reference can be made only by a comparison of clearances (*i.e.*, rates of excretion relative to plasma concentration) of various substances under various physiological conditions. The use of inulin as the standard of reference for the measurement of the rate of filtration of plasma water rests upon good experimental evidence, so far as the normal human kidney is concerned (13).<sup>1</sup>

In the diseased kidney, however, an increase in permeability of the tubule may permit the escape of water from the tubular urine without permitting the escape of inulin; and, conversely, a decrease in permeability of the glomerular membranes may retard the filtration of inulin without proportionally retarding the filtration of water;<sup>2</sup> in either case, the differential movement

of water cannot be detected by changes in  $C_{IN}$ , although, if the alternative possibility can be ruled out, it may conceivably be deduced by the comparison of  $C_{IN}$  with the clearance of some smaller molecule.<sup>3</sup>

Lastly, it is conceivable that filtration may continue in a glomerulus after the attached tubule has become separated from its collecting duct, or that the tubule may become so permeable that all the constituents in the glomerular filtrate, including inulin, escape into the interstitial fluid rather than the urine.  $C_{IN}$  represents only such inulin as is passed into the bladder and will reveal nothing of these circumstances. Whatever significance the complete local reabsorption of glomerular filtrate may have in renal pathology, it remains beyond the possibility of examination so long as only the total urine is available for analysis.

#### *Renal blood flow and diodrast clearance*

The diodrast clearance may be defined as the virtual volume of plasma completely cleared of diodrast by the renal parenchyma per unit of time. On the assumption that the diodrast is completely removed from all plasma presented to active excretory tissue, it may be identified as the actual plasma flow to this tissue. This identification, however, does not include plasma (or blood) which perfuses inert tissue, and consequently  $C_D$  bears no certain relation to the total renal blood flow. But inasmuch as  $C_D$  approaches in magnitude the total renal blood flow, it affords in effect a method of following the latter, but subject only to the necessary qualifications with respect to the completeness of extraction from the total renal blood.

(oncotic plus capsular pressure) is not reached, or is not approached as closely as in the normal glomerulus (11).

<sup>3</sup> The parallel behavior of urea and inulin in all stages of diffuse glomerular nephritis indicates that the reduced excretion of both substances is primarily attributable to the obliteration of the filtering bed rather than increased back-diffusion of urea (1).

<sup>1</sup>  $C_{IN}$  may be calculated as cc. of plasma water instead of cc. of plasma, but this is superfluous where other analyses and calculations refer to cc. of plasma, and where it is convenient to speak of volumes of plasma filtered.

<sup>2</sup> A reduction in the filtration of water resulting from decreased permeability of the glomerular membranes requires that in the glomerular capillaries equilibrium between the hydrostatic pressure and the opposed forces

Similarly the identity of the inulin and hexitol clearances in women with pre-eclampsia (13) argues against a significant decrease in glomerular permeability in a disease where thickening of the glomerular membranes is frequently observed. There is here no reason to suspect that a precisely compensating increase in tubular permeability has obscured a decrease in glomerular permeability, since tubular function is apparently not disturbed (14).

In this connection we may first note the consequences of the appearance in the renal pattern of impotent nephrons. The efferent blood from the glomerulus attached to the impotent tubule—blood which once was cleared by this tubule—will be available for clearance by such normal tubules as are located within the maximal sphere of diffusion of diodrast, etc., or circulation of the interstitial fluid.

With regard to diffusion, a concentration gradient will be established between the capillary of the impotent tubule and such normal tubules as are excreting diodrast; at a constant plasma concentration of diodrast, the time required for molecules to move the length of this gradient may be neglected, and the radius of diffusion may be conceived to be limited only by some actual interruption of the diffusion free-way. Conceivably, the diodrast cleared by a particular tubule may diffuse out of a remote capillary.

With regard to the circulation of interstitial fluid, it will be noted that out of 135 cc. of glomerular filtrate formed each minute, the greater fraction is reabsorbed by the tubules and must pass across the interstitial space to the capillary before it re-enters the blood. It cannot be imagined that this movement of water occurs along the shortest possible point-to-point route; on the contrary, there must be a considerable streaming between or along the tubules which is aided by the arterial pulse. This streaming will not only accelerate the movement of diodrast from capillary to excretory tubule but it may also increase the radius of clearance to a much larger area than diffusion alone would permit. Although diffusion and the circulation of renal interstitial fluid must play an important rôle in normal renal function,<sup>4</sup> their significance is ex-

perimentally more evident in the diseased kidney.

The increased volume of blood which is made available for clearance by the formation of impotent nephrons will appear in clearance tests as an apparent hyperemia of the residual functional tissue (increase in  $C_D$  relative to  $Tm_D$ ); but unlike the hyperemia resulting from either dilatation of the renal arterioles or increased perfusion pressure, this apparent hyperemia may not afford the normal tubules a proportionately increased supply of oxygen, etc., and it seems advisable to distinguish it from true hyperemia (vascular dilatation) by qualifying it as a vicarious hyperemia.

It is conceivable that loss of excretory activity may occur in large areas of a kidney, in single tubules, or in alternate cells of a tubule; in any of these instances, the extraction ratio of diodrast in the blood which once was cleared by the now defunct tissue (unless it is anatomically available for vicarious clearance by residual functional units) will decrease from its high normal value (which we nominally take as 1.0) and ultimately fall to zero. Thus, in considering the diseased kidney as a whole, the overall extraction ratio may be expected to have any value between 1.0 and 0.0, and  $C_D$  will have a very uncertain relation to the total renal blood flow.<sup>5</sup> This circumstance does not, however, impair the usefulness of the clearance method; on the contrary, it is under these conditions that it acquires a unique physiological significance.

It will perhaps aid the reader to visualize the following argument if, as an extreme example, an inert cannula is imagined to be inserted between the renal artery and the renal vein of a normal kidney so that some large fraction of the renal arterial blood passes directly to the renal vein by way of this cannula. Since the clearance method depends upon the presence of living excretory tissue to remove diodrast (or some other suitable substance) from the blood and excrete it in the urine, this method can never discover how much blood passes from renal ar-

which are known about this process indicate that the major movement of fluid is from the medulla towards the periphery of the kidney (3).

<sup>5</sup> This circumstance was recognized in our original description of the diodrast clearance method (12) and was the basis of the definition of this clearance as the *effective* renal blood, i.e., the virtual volume of blood which is completely cleared of diodrast.

<sup>4</sup> The fact that an average plasma extraction ratio of 0.74 (15) or 0.85 (2) is observed in the explanted dog kidney is in itself unexpected, since some postglomerular blood must pass rapidly to medullary tissue which is supposed to be reabsorptive rather than excretory; physical diffusion and circulation of interstitial fluid probably obviate the local distribution of blood and aid in the maintenance of this high extraction ratio. Any circumstance which would restrict the movement of interstitial fluid (increased intrarenal pressure, perinephritis, etc.) would be equivalent in effect to a reduction in actual blood flow to such areas in the kidney as might be dependent for their perfusion on the circulation of interstitial fluid rather than on a direct blood supply. The meager facts

tery to renal vein through the cannula. Nor will the cannula begin to excrete diodrast when the plasma concentration is raised; that is, it will not only fail to contribute to  $C_D$  such diodrast as is carried by the blood passing through it at low concentrations, but it will also fail to contribute diodrast to  $Tm_D$  when this is measured at high plasma concentrations. The same consideration applies to blood exclusively perfusing inert tissue; <sup>6</sup> if particular diodrast molecules are not brought into effective juxtaposition with functional excretory tissue for clearance at a low plasma concentration, in general <sup>7</sup> they will not be made available for clearance simply in consequence of increasing the plasma concentration. And any tissue incapable of excreting diodrast

<sup>6</sup> The term "exclusively perfusing" excludes diffusion through and circulation of the interstitial fluid, while the term "inert tissue" excludes normal tubular tissue which is inactive simply in consequence of ischemia or because it is presented only with blood which has previously been cleared of diodrast.

<sup>7</sup> It is conceivable that a decrease in permeability of the capillaries, interstitial tissue or tubule cell, or some change in the tubular excretory mechanism itself, could retard the movement of diodrast from blood to urine; since the blood is available for clearance in the peritubular capillary for only a brief period, this retardation would lower the percentage of the diodrast removed during its passage down this capillary. But whether the retarding factor is conceived as a positive barrier (*e.g.*, impermeable connective tissue) or a negative fault (*e.g.*, failure of the tubule cell to handle all molecules available to it), it would be expected that the retardation would operate on any and all molecules of diodrast with statistical indifference, with the result that the clearance probabilities for any particular molecule would not be increased by increasing the number of molecules.

Exceptions to the above statement are conceivable: one might imagine that at a low concentration so large a fraction of diodrast was absorbed on the plasma protein that escape from the capillary by diffusion was greatly retarded and the extraction ratio correspondingly reduced; if now at some higher concentration the plasma proteins became saturated, the diffusion of diodrast from the capillary, and consequently the extraction ratio, would be increased. Since protein binding is a function of diodrast concentration, this particular situation, chosen for illustration only, is not to be expected in practice. Whether or not as a result of disease a change in the kinetics of the tubular excretory process could have this consequence is undetermined, but it would seem that the problem can be examined by the progressive elevation of the plasma diodrast level. Such titrations as have been made by us to date have supplied no evidence of such a change.

at low plasma levels will not acquire excretory capacity by virtue solely of an increased concentration of diodrast. We may generalize from the above by saying that where the plasma concentration is the only changing factor, inert tissue is excluded from both  $C_D$  and  $Tm_D$ ; hence in the measurement of  $C_D$  and  $Tm_D$  the same vascular and interstitial channels are involved, and in turn  $C_D$  and  $Tm_D$  refer to the same nephrons or other units of excretory activity. Whether  $Tm_D$  is conceived in terms of entire nephrons, individual cells or hypothetical excretory units of minimal dimensions, is immaterial: the ratio  $C_D/Tm_D$  expresses the virtual quantity of plasma completely cleared of diodrast per unit quantity of the excretory tissue which is effecting this clearance.  $C_D$  here has the same definition and the same significance as in the normal kidney in which, as emphasized above, there is already some inert tissue and some uncleared blood; if in disease some of the blood presented to a particular nephron or a particular tubule cell escapes uncleared, the significance of the ratio remains unchanged.

Thus a reduction in the overall extraction ratio of diodrast in the diseased kidney does not seriously impair the usefulness of the clearance method. On the contrary, this method is the only one capable of determining the volume of blood cleared by such functional excretory units as are inextricably mixed with scar tissue, tubular detritus, connective tissue, etc., a datum upon which no information could be gained by the measurement of the total renal blood flow. When the diodrast clearance is referred to the total quantity of available functional tissue, *i.e.*, by utilization of the ratio  $C_D/Tm_D$ , a datum is obtained which in most instances will be qualitatively and quantitatively comparable with observations made on the normal kidney. Even in normal subjects,  $C_D$  should be related to  $Tm_D$  in order to take into account the varying quantities of excretory tissue which may be expected to occur in different individuals.

#### SUMMARY

The interpretation of clearance methods (the inulin and diodrast clearance, and diodrast  $Tm$ ) in the diseased kidney is discussed.

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# EFFECTIVE RENAL BLOOD FLOW IN SUBJECTS WITH ESSENTIAL HYPERTENSION<sup>1,2</sup>

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Sixty patients with well-established essential hypertension form the basis of this report. These patients were selected from the Hypertension and Nephritis Clinic of the New York University Clinic. A few subjects with radiologic evidence of uropathology are included, but none who have evidence of glomerular nephritis or other specific renal disease.

A major difficulty in a problem such as is considered here is that the investigator cannot readily observe a particular patient pass from health through various stages of the disease, nor yet pass from any stage of the disease back into health. The perturbations of function in essential hypertension are so insidious in onset and generally so slow in progress that they can be discovered only by a comparison of subjects in many phases of the disease with a number of normal subjects. Although this comparative method contains certain obvious dangers, we believe that the facts presented in this study demonstrate significant differences in renal function in hypertensive and normal subjects.

Pertinent clinical data are summarized in Table I, and the important data on renal function in Tables II and III. The only points needing explanation are that the blood pressures in Tables II and III represent the average of several values observed by the auscultatory method during the measurement of the diodrast clearance. Each datum on the inulin clearance and diodrast clearance is the average of three or more urine collection periods, each datum on diodrast  $Tm$  the average of five periods. Additional basal observations, comparable with those of Table II, are presented in Table III for purposes of reference in examining the effects of renal hyperemia. The data in Table II and the

control data in Table III refer to the basal condition, uncomplicated by any therapy.

We have previously shown the advantage of comparing renal function in various normal subjects in terms of their respective values of diodrast  $Tm$  (9), and have amplified the reasons for this method of analysis, in respect to the diseased kidney, in the preceding paper (12). This relative method of analysis is followed here, the data on hypertensive subjects being presented against a statistical background afforded by the behavior of the normal kidney. The data for this purpose are drawn from Table IV of a previous paper (9) where men and women are treated as one series, sex differences being negligible.

## 1. Diodrast clearance and diodrast $Tm$

Figure 1 shows the diodrast clearance ( $C_D$ ) in relation to diodrast  $Tm$  ( $Tm_D$ ). Each subject is recorded once only, the average basal values of  $C_D$  and  $Tm_D$  taken from Tables II and III being used. In normal subjects (9) the ratio,  $C_D/Tm_D$ , has a mean value of  $13.4 \pm 1.4$ ; this mean normal value is represented by the solid line,  $M$ , the lines above and below  $M$  denoting multiples of the standard deviation. The ellipse is calculated to contain 70 per cent of the normal observations and actually contains 72 per cent.<sup>3</sup>

<sup>3</sup> This ellipse and the corresponding ellipse in Figure 2 were not calculated in the paper in which the normal data were presented.

The area on a scatter diagram within which we may theoretically expect 70 per cent of the observations to fall by chance alone is an ellipse formed by the equation

$$x^2 = \left( \frac{x^2}{\sigma_x^2} - \frac{2rxy}{\sigma_x\sigma_y} + \frac{y^2}{\sigma_y^2} \right) \frac{1}{1-r^2},$$

where  $x^2$  is taken from recorded  $x^2$  tables (10) and is determined by  $P$ , the proportion of observations which may be expected to fall outside the ellipse by chance alone, and by  $n$ , the degrees of freedom in the system. Here  $P$  is 0.30 (i.e., 70 per cent within the ellipse) and  $n = 2$ ; hence,  $x^2 = 2.408$ . In the above equation  $x$  is the distance from

<sup>1</sup> Aided by a grant from the Commonwealth Fund.

<sup>2</sup> Preliminary communications on this subject have appeared elsewhere (8, 16).

If we were to start with a normal kidney in which  $C_D/Tm_D = 13.4$  and were to reduce the quantity of tubular excretory tissue and the renal blood flow by proportional amounts, we would pass down the line  $M$  until we reached the intersection of the zero ordinates. This is not to imply that the statistical regression line relating  $C_D$  to  $Tm_D$  in normal subjects extrapolates to zero, for it does not; the concept of proportional regression is merely an artifice convenient to functional interpretation. A variation of mean  $C_D/Tm_D \pm 2\sigma$  ( $13.4 \pm 2.8$ ) should contain 95 per cent of the normal observations, and it is in keeping with the artifice of proportional regression to conceive that any distribution of the hypertensive data disproportionate with this statistical expectation is indicative of significant functional changes in  $C_D$ ,  $Tm_D$  or both.

The following facts are to be noted in Figure 1: With the exception of three subjects (F. O., R. D., and G. T.),  $Tm_D$  is below the mean of the normal value (51.6 mgm. of iodine per minute) and ranges from slightly subnormal to very low values. As shown in Table I, the lowest values of  $Tm_D$  are found in subjects with advanced retinopathy and significant proteinuria. In the latter instances it is evident on a statistical basis that impairment of tubular function, as judged by the loss of the capacity to excrete diodrast under conditions of saturation, has occurred. Whether or not  $Tm_D$  has been reduced in those subjects in whom this value approaches the normal value cannot be answered from statistics alone, but it is inferred that such is the case,

$mx$  along the  $x$  axis,  $y$  is the distance from  $my$  along the  $y$  axis,  $\sigma_x$  and  $\sigma_y$  are the standard deviations of the distribution in the  $x$  and  $y$  direction, respectively, and  $r$  is the coefficient of correlation.  $x$  is determined for various values of  $y$  by resolving the above equation in the quadratic:

$$x = \frac{-ay}{2} \pm \sqrt{\left(\frac{a^2}{4} - b\right)y^2 - c}$$

and writing

$$a = -\frac{2r\sigma_x}{\sigma_y}$$

$$b = \frac{\sigma_x^2}{\sigma_y^2}$$

$$c = -(1 - r^2)x^2.$$

An ellipse on a scatter diagram within which 70 per cent of the observations may be expected to fall by chance corresponds roughly to a distance from  $-1\sigma$  to  $+1\sigma$  on a linear scale for one variable (68 per cent of the observations).

TABLE I

Clinical data on hypertensive subjects

(The subjects are arranged in order of decreasing diodrast  $Tm$ , this value being the average of all observations under basal conditions.)

Subject	Diodrast <i>Tm</i>	Range of blood pressure during hospitalization	Retina*	Enlarged heart	Proteinuria	Hematuria	Anemia†	Necropsy
	(mgm. iodine per minute)							
R. D.	56.3	168/120-174/124	1	Y	0	0	N	N
G. T.	53.4	124/ 88-130/104	0	N	0	0	N	N
F. O.	53.2	106/ 80-112/ 99	0	N	0	0	N	N
C. V.	48.9	142/ 86-194/150	1	0	0	0	N	N
G. G.	48.3	150/ 95-165/120	1	0	0	0	N	N
M. J.	46.8	152/ 80-174/110	1	N	0	0	N	N
A. M.	46.2	160/100-180/110	0	N	0	0	N	N
A. Mc.	45.1	170/106	1	N	0	0	N	N
M. G.	44.7	134/ 90-180/120	1	Y	0	0	N	N
C. T.	44.7	158/ 84-210/108	1	N	0	0	N	N
M. C.	44.3	164/112-192/126	1	N	0	0	N	N
S. K.	42.7	160/110-210/134	1	Y	+	0	N	N
A. N.	41.9	138/ 78-158/100	2	N	0	0	N	N
M. A.	40.9	178/120-226/140	1	Y	0	0	N	N
E. G.	40.1	160/102-190/120	1	Y	0	0	N	N
L. Js.	40.1	112/ 80-182/124	1	Y	0	0	N	N
R. Mc.	39.0	165/105-230/128	1	Y	0	0	N	N
M. Jo.	38.6	132/100-170/124	1	N	0	0	N	N
A. G.	38.6	192/ 90-230/130	2	Y	0	0	N	N
M. M.	37.9	148/110-160/110	1	N	+	0	N	N
V. V.	37.5	134/ 88-186/116	1	N	0	0	N	N
C. B.	36.7	116/ 80-196/120	0	N	0	0	N	N
S. W.	36.5	190/110-220/128	1	Y	0	0	N	N
E. H.	36.2	154/ 94-190/130	2	Y	0	0	N	N†
H. N.	35.4	148/100-164/120	1	N	0	0	N	N
M. T.	35.1	160/104-180/124	1	Y	0	0	N	N
F. S.	34.4	175/ 95-208/114	1	Y	0	0	N	N
R. Y.	34.2	180/125	1	Y	+	0	N	N†
K. S.	33.5	210/130-260/180	1	Y	0	0	N	N
L. S.	32.8	190/120-240/150	1	N	0	0	N	N
R. L.	32.6	200/125-240/140	1	Y	0	0	N	N
A. Mg.	32.6	160/ 98-196/110	1	Y	0	0	N	N
R. La.	32.6	130/ 80-170/105	1	N	0	0	N	N
U. B.	32.5	190/118-200/140	1	Y	0	0	N	N
F. K.	31.6	144/ 92-170/112	1	N	0	0	N	N
W. N.	30.9	162/110-238/170	1	Y	0	0	N	N†
J. O.	30.5	152/106-230/146	2	Y	0	0	N	N
L. J.	30.1	102/ 76-178/128	0	Y	0	0	N	N
L. K.	29.5	160/ 90-224/128	1	N	0	0	N	N†
E. D.	28.9	154/110-210/140	1	Y	0	0	N	N
E. Wa.	28.2	154/108-216/126	2	N	0	0	N	N†
A. B.	27.1	154/104-180/130	1	Y	0	0	N	N
M. P.	26.6	190/110-230/130	2	Y	0	0	N	N
H. U.	26.5	164/ 90-230/134	2	N	+	0	N	N
D. C.	25.7	132/ 78-200/120	2	Y	0	0	N	N
T. T.	24.9	174/102-180/112	1	Y	0	0	N	N
G. F.	24.6	180/118-230/118	1	Y	0	0	N	N
G. L.	22.7	148/110-270/170	1	N	0	0	N	N
J. M.	19.8	194/162-220/152	3	Y	++	+	Y	Y
J. M.	16.6	220/130-280/180	3	Y	++++	+	Y	Y
G. H.	14.8	222/ 96-244/132	3	Y	++++	+	Y	Y
H. G.	12.3	180/100-250/148	3	Y	++++	+	Y	N†
E. J.	8.9	204/130-270/160	3	N	++	+	Y	N†
J. Br.	4.6	166/112-202/145	3	Y	+++	+	Y	N†
J. L.	4.3	170/ 95-210/110	3	Y	+++	+	Y	Y
N. S.	3.0	164/ 96-178/110	3	Y	+	+	Y	Y

BILATERAL SYMPHRECTOMY

B. Fo.	37.0	140/ 90-210/120	3	Y	+	Y	N	N
W. Mc.	27.2	230/155	1	Y	0	0	N	N

UNILATERAL NEPHRECTOMY

J. G.	37.2	150/ 88-154/ 94	1	N	0	0	N	Y
W. S.	33.8	164/108-298/160	1	Y	Y	Y	Y	Y

\* 0 = negative; 1 = vascular changes; 2 = 1 + retinopathy; 3 = 2 + papilledema.

† Less than 3 M. or less than 70 per cent hemoglobin.

‡ Deceased.

TABLE II

*Basal observations on hypertensive subjects*

(Columns 4, 5, 6, 7 and 10 are corrected to 1.73 sq. m., column 10 corrected to 98.5° F. (15). Columns 11 and 12 are averages of columns 6 and 4 divided by the averages of column 10. Additional basal data given in Table III are included in above averages.)

Subject	Date	Average blood pressures during $C_D$ determination	Plasma clearances			Effective blood flow	Filtration fraction	Temperature	$T_{MD}$	$C_D/T_{MD}$	$C_{IN}/T_{MD}$
			Inulin	Phenol red	Diodrast						
			cc. per minute	cc. per minute	cc. per minute	cc. per minute	per cent	° F.			
G. G.	December 6, 1937		116		503	818	23.1	99.6	52.8		
	February 16, 1938		149	305	506	830	29.5	99.0	48.6		
	October 10, 1938*	148/98	106	296	428	652	24.8	99.0	44.3		
M. G.	October 21, 1938	148/100	115	276	441	689	26.1	98.8	47.5	9.7	2.51
	December 17, 1937		124	327	589	1027	21.1				
	December 20, 1937		122	368	576	1027	21.2	99.5	53.2		
M. J.	February 9, 1938		123	317	521	921	23.6	98.6	41.3		
	February 26, 1941	152/100	121					99.0	40.7	12.5	2.72
	May 20, 1940†	150/94	139	369	536	862	25.9	98.5	46.8	11.4	2.97
A. M.	February 25, 1938		135	398	632	1176	21.4				
	March 2, 1938		127	335	584	1003	21.7		46.2	13.2	2.84
	April 22, 1940†	198/118	98.4	261	336	544	29.3	98.0	45.1	7.4	2.18
A. Mc.	December 4, 1939	206/106	105		510	832	20.6	98.4	44.7	11.4	2.35
C. T.	March 29, 1940†	180/124	149	344	565	835	26.4	98.0	44.3	12.8	3.36
M. C.	October 7, 1940†	196/130	99.6		358	602	27.8	98.6			
S. K.	November 8, 1940†	142/98							42.7	8.4	2.33
A. N.	November 4, 1940†	158/100	120		452	717	26.6	99.7	41.9	10.8	2.86
M. A.	December 27, 1937		142	316	556	1035	25.5				
	December 30, 1937		117	356	598	1075	19.6	99.0	41.9		
	November 6, 1939	204/128	151		490	823	30.8	98.6	41.0		
M. Jo.	November 15, 1939	194/126	122		524	952	23.3	99.2	40.5		
	December 6, 1939	194/128	117		445	686	26.3	99.6		12.7	3.16
	February 21, 1938		118	294	485	824	24.3	98.6	41.1		
E. G.	March 21, 1938		132	402	531	848	24.9	99.6	41.3		
	April 4, 1941	194/120	105		461	752	22.8	99.4	35.9		
	May 7, 1941	152/198	83.6		570	877	14.7	99.2	45.1	12.5	2.69
R. Mc.	December 8, 1937		130	346	475	1002	27.4	98.4	43.4		
	December 22, 1937		113	251	448	999	25.2				
	November 18, 1940†	212/110	94.8		415	728	22.8	98.6	36.9		
A. G. §	December 9, 1940†	200/120	108		488	856	22.1	98.6	41.1	13.6	2.60
	October 4, 1940†	264/126	71.9		354	580	20.3	98.8	38.6	9.2	1.86
	December 19, 1940†	136/90	106		568	1062	18.7	98.8	38.3	14.8	2.77
L. Js.	April 8, 1940†	200/146	121	297	380	735	31.8	97.2	37.9	10.0	3.19
M. M.	April 22, 1940	140/92	132		732	1118	18.1	97.8	37.5	19.5	3.44
V. V.	May 17, 1940	128/92	101		392	652	25.8	99.9	35.4	11.1	2.85
H. N.	March 3, 1941†	166/108	127		579	982	21.9	98.6	35.1	16.5	3.62
M. T.	November 29, 1940	200/110	70.5		366	666	19.3	98.2	36.5	10.0	1.93
S. W.	November 14, 1940†	204/120	142		504	840	28.2	98.0	34.4	14.6	4.12
F. S.	March 25, 1938		105	230	323	588	32.5	98.6	34.6		
R. Y.	April 4, 1938		107	216	324	568	33.0	98.4	33.7	9.5	3.10
K. S.	May 12, 1941†	186/108	136		388		35.1	98.5	33.5	11.6	4.06
C. B.	May 16, 1941	124/75	115		542	822	21.2	98.8	36.7	14.8	3.13
L. S.	April 15, 1940†	250/120	102	245	436	799	23.4		32.8	13.3	3.11
A. Mg.	September 27, 1940†	200/120	112		419	746	26.7		32.6	12.9	3.43
R. L.	November 11, 1940	166/130	112		500	910	22.4	98.8	32.6	15.3	3.43
U. B.	May 19, 1939	198/130	104		416	695	25.0	98.3	32.5	12.8	3.20
J. O.	March 12, 1941	210/138	79.8		347	691	23.0	98.4	29.5	11.8	2.70
F. K.	January 10, 1938		126	304	530	1080	23.8				
	January 24, 1938		117	282	513	930	22.8	98.4	34.0		
	February 2, 1938		126	260	545	1002	23.1				
	March 9, 1938		107	233	487	934	22.0	99.0	29.9		
	May 17, 1938	156/110	92.4	240	473	942	19.5				
	October 26, 1938	154/110	99.4	265	530	1060	18.7				
	November 2, 1938	148/100	96.3	244	497	904	19.4				
	November 11, 1938	148/100	88.2	197	457	774	19.3				
	May 27, 1940†	160/112	131	328	633	1179	20.7		31.4	16.3	3.44

TABLE II—Continued

Subject	Date	Average blood pressures during $C_D$ determination	Plasma clearances			Effective blood flow	Filtration fraction	Temperature	$T_{mD}$	$C_D/T_{mD}$	$C_{IN}/T_{mD}$
			Inulin	Phenol red	Diodrast						
			cc. per minute	cc. per minute	cc. per minute	cc. per minute	per cent	° F.			
W. N.	January 7, 1938		84.8	180	314	575	27.0	100.0	30.9	10.2	2.74
L. J.	October 19, 1938	160/110	97	218	275	500	35.3	101.5	29.1		
	January 3, 1939 <sup>  </sup>	144/98	111	252	421	668	26.4	99.3	28.2		
	January 18, 1939	144/110	124	321	573	895	21.6	99.2	34.3		
	March 22, 1939	146/110	94		391	610	24.0	99.5	30.3		
	December 2, 1940 <sup>†</sup>		118		419	737	28.2	98.5	29.8	13.7	3.59
L. K.	January 10, 1938		104	191	323	552	32.2				
	January 19, 1938		88.8	150	267	435	33.2	98.6	28.6		
	March 18, 1938		120	171	292	468	41.1	99.4	30.3		
	May 20, 1938	230/130	115	219	333	571	34.5				
	May 25, 1938		86.9	157	295	482	29.5			10.3	3.49
E. D.	June 24, 1940 <sup>†</sup>		102		425	639	24.0	98.5	30.6	14.7	2.96
E. Wa.	April 1, 1940 <sup>†</sup>		81		308	540	26.3	98.5	29.6	10.9	2.82
A. B.	April 15, 1940		68.2		331	535	20.6	98.4	27.1	12.2	2.52
M. P.	December 3, 1937		69.1	191	338	537	20.4	98.6	29.7		
	March 14, 1938		75.4	185	268	435	28.1	99.4	23.5	11.4	2.72
H. U.¶	December 1, 1937		91.4	246	532	960	17.2				
	December 13, 1937		91.8	225	475	763	19.3	99.0	26.6		
	March 23, 1938		110	259	529	896	20.8				
	October 4, 1938 <sup>†</sup>	210/124	105	231	497	822	21.1	99.0	24.2		
	October 27, 1938	196/122	99.0	246	503	889	19.7	99.0	26.5		
	November 17, 1938	192/110	108	221	521	829	20.7			19.8	3.91
T. T.	March 19, 1941	200/110	61.9		280	470	22.1	98.6	24.9	11.2	2.49
D. C.	November 3, 1940	176/100	105		530	888	19.8	98.8	25.7	20.6	4.08
G. F.**	November 25, 1940 <sup>†</sup>	148/100	59.8		303	517	19.7	98.0	24.6	12.3	2.43
J. M.	March 14, 1941	204/110	90.7		375	744	24.2	98.4	22.9	16.4	3.96
G. L.††	December 23, 1940 <sup>†</sup>	170/134	68.4		241	518	28.4	98.3	22.7	10.6	3.01
G. H.	February 11, 1938		40.7	86.6	168	272	24.2	99.0	14.3		
	March 4, 1938		39.2	88.6	154	245	25.5	99.0	15.2	10.9	2.71
E. J.	May 24, 1938		24.5	45.1	79.8	127	30.7	99.0	8.9	9.0	2.87
J. Br.	February 26, 1940	200/156	10.0		38.3	85.2	26.1	98.4	4.6	8.3	2.12
J. L.	December 29, 1937		20.6	32.8	69.6	102	29.6				
	January 5, 1938		16.6	29.5	60.8	88	27.3				
	January 17, 1938		23.8	31.6	77.6	109	30.7	98.6	5.5		
	March 16, 1938		20.7	18.4	42.2	56.2	49.0	98.6	3.0	14.7	4.81
N. S.	February 7, 1938		7.1	10.0	43.0	61.0	16.5	98.8	3.4		
	March 11, 1938		6.9	9.0	31.3	41.7	22.0	98.6	2.6	12.4	2.33

## BILATERAL SYMPATHECTOMY

W. Mc.††	February 28, 1940	188/128	105		339	703	31.0	99.4	27.2	12.5	3.86
B. Fo.§§	May 13, 1940	154/106	88.4		462	683	19.1	98.6	37.0		
	June 3, 1940 <sup>†</sup>	174/120	122	271	396	618	30.8	98.5	37.0		
	June 17, 1940 <sup>†</sup>	170/120	107	274	438	683	24.4	98.5		11.7	2.86

## UNILATERAL NEPHRECTOMY

J. G.	February 12, 1940	162/108	83.2		354	622	23.5	99.2	37.2		
	February 16, 1940	150/80	89.2		416	885	21.4	99.2		10.3	2.32
W. S.¶¶	October 31, 1938	142/82	122		592	1037	20.6		33.8	17.5	3.61

\* G. G. Left renal omentopexy on June 14, 1938.

† Unilateral method.

‡ S. K. No change in renal blood flow in sitting position.

§ A. G. Studied ten days after recovery from congestive heart failure.

|| L. J. Right nephropexy on October 12, 1938, for costovertebral pain and hematuria.

¶ H. U. Left omentopexy, June 14, 1938. Attempt at right omentopexy on July 10, 1939, abandoned because of inability to bring omentum to kidney.

\*\* G. F.  $T_{mD}$  done in sitting position.

†† G. L. Denervation of right renal pedicle, June 4, 1940. On December 23, 1940, right kidney was functionless.

‡‡ W. Mc. Bilateral sympathectomy in June, 1938.

§§ B. Fo. Bilateral sympathectomy, left side on May 18, 1939, and right side on June 9, 1939.

|||| J. G. Right nephrectomy on August 30, 1939. Diagnosis, atrophy of kidney with hydronephrosis.

¶¶ W. S. Right nephrectomy in 1936. Diagnosis, renal tuberculosis. Not hypertensive.

TABLE III

*Observations on hypertensive subjects during induced hyperemia*

(Compared with last preceding observation made under basal conditions, usually one week previously. Asterisk marks the hyperemic study.)

Subject	Date		Mean blood pressure during $C_D$ determinations	Plasma clearance		Effective blood flow	Filtration fraction	Rectal temperature	$T_{MD}$	$D\text{-load}/T_{MD}$	$C_D/T_{MD}$	$\Delta C_{IN}$	$\Delta C_D$	$\Delta T_{MD}$
				Inulin $C_{IN}$	Dio-drast $C_D$									
				cc. per minute	cc. per minute	cc. per minute	per cent	° F.	mgm. iodine per minute			per cent	per cent	per cent
R. D.	June 24, 1940		168/120	150	726	1088	20.7	99.0	56.3	2.41	12.9			
	*June 28, 1940		140/92	136	1000	1494	13.6	99.3	54.7	5.14	18.3	-9	+38	-3
F. O.	June 17, 1940		118/74	130	768	1227	16.9	99.2	53.2	1.65	14.4			
	*June 26, 1940		112/64	117	1097	1797	10.7	99.2	53.8	3.90	20.4	-10	+43	+1
C. V.	May 29, 1940		216/116	96.8	406	664	23.8	98.6	48.9	1.63	8.3			
	*June 5, 1940		176/96	86.3	610	931	14.1	98.5	34.9	5.05	17.5	-11	+50	-29
M. G.	January 6, 1939			111	555	925	20.0	98.8	43.5	1.50	12.8			
	*January 11, 1939		136/88	114	1010	1683	11.3	99.6	45.6	3.50	22.2	+3	+80	+5
M. Jo.	May 1, 1939		144/98	114	556	785	20.5	98.8	29.8	1.75	18.6			
	*May 8, 1939		130/88	106	717	1094	14.8	99.6	34.0	6.35	21.1	-7	+37	+14
E. G.	January 12, 1939		210/120	130	492	910	26.4	98.4	36.9	1.80	13.3			
	*January 16, 1939		170/90	116	886	1610	13.4	99.8	39.2	4.50	22.6	-11	+88	+6
V. V.	April 5, 1940		148/106	125	775	1215	16.1	98.6	37.5	4.97	20.6			
	*April 30, 1940		144/86	124	1318	2042	9.4	98.4	43.0	7.56	30.6	±0	+75	+15
J. O.	June 10, 1940		188/132	93.9	408	752	23.0	98.6	32.4	2.47	12.6			
	*June 19, 1940		186/126	88.3	685	1262	13.0	99.3	33.3	3.73	26.5	-6	+68	+3
F. K.	March 13, 1939		168/102	121	604	1105	20.0	98.8	30.9	9.8	19.6			
	*March 20, 1939		164/90	116	914	1375	12.7	99.4	29.6	13.3	23.1	-4	+72	-4
	March 1, 1940		158/114	140	579	1108	24.2	99.0	32.0	2.91	18.1			
	*May 24, 1940		166/120	122	752	1400	16.2	99.3	40.8	3.72	18.4	-13	+42	+18
L. J.	March 27, 1939		134/90	120	471	785	25.5	99.3	29.0	3.87	16.2			
	*April 3, 1939		128/90	116	622	1056	17.3	99.8	31.4	4.71	19.8	-3	+46	+8
E. D.	May 27, 1940		196/142	69.3	386	601	18.0	98.6	27.1	3.21	14.2			
	*June 3, 1940		184/118	63.1	571	857	11.1	98.7	27.8	4.35	20.5	-9	+41	+3
E. Wa.	April 8, 1940		174/108	78.0	344	583	22.7	98.3	26.8	3.72	12.8			
	*April 24, 1940		178/108	80.4	827	1343	9.7	98.6	32.0	7.33	25.8	-3	+157	+19
A. B.	April 1, 1940		170/120	77.2	393	660	19.6	98.1	27.1	3.85	14.5			
	*April 26, 1940		174/118	70.8	659	1096	10.7	98.3	25.9	5.92	25.4	-8	+82	-4
H. U.	March 29, 1939		198/112	111	544	899	20.4	99.3	24.4	9.73	22.3			
	*April 10, 1939		172/102	103	567	831	18.2	98.5	22.6	10.58	25.1	-7	+8	-7
	*April 17, 1939		160/88	117	850	1315	13.8	100.0	24.5	14.08	34.7	+5	+62	±0
	March 8, 1940		172/104	92.6	582	956	15.9	98.8	31.0	3.64	18.8			
	*May 1, 1940		152/90†	48.5	394	606	12.3	99.0	35.0	3.07	11.3	-59	-25	+13
R. L.	March 10, 1939		230/132	145	570	976	25.4	99.0		2.43				
	*March 17, 1939		182/110	118	758	1330	21.1	99.0	41.0	7.91	18.5	-19	+33	
J. M.	May 6, 1940		232/158	51.0	156	252	32.7	98.4	16.6	2.76	9.4			
	*May 15, 1940		248/162	46.3	152	241	30.4	98.7	10.9	5.64	13.9	-9	-3	-34
H. G.	April 19, 1939		240/160	63.2	207	362	30.5	100.0	12.3	5.77	17.0			
	*April 24, 1939		236/136	67.1	241	400	27.8	99.0	14.1	4.43	17.1	+6	+17	+15
	*May 12, 1939		248/142	60.8	212	348	28.8	99.0	16.4	3.60	12.9	-4	+2	+33
E. H.	May 3, 1940		230/138	76.4	296	539	25.8	99.1	36.2	2.57	8.2			
	*May 8, 1940		206/122	86.2	654	1120	13.2	99.5	28.8	7.11	22.7	+13	+121	-21

AFTER UNILATERAL SYMPATHECTOMY ON LEFT SIDE, MAY 20, 1940†

	*June 12, 1940	210/128	R 41.8 L 42.2	284 280	415 409	14.7 15.1	98.8					+10 +10	+92 +89	
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BILATERAL SYMPATHECTOMY

W. Mc.	February 28, 1940	188/128§	105	339	703	31.0	99.5				12.5			
	*June 7, 1940	198/130	115	443	832	25.9	99.4	27.2	1.20	16.2	+10	+31		
B. Fo.	May 13, 1940	156/108	88.4	462	683	19.1	98.6	37.0	4.29	12.5				
	*May 20, 1940	146/90	83.2	628	925	13.2	99.4	41.8	2.7	15.0	-6	+45	+13	

† During pyrogenic reaction symptoms of circulatory failure occurred. Recorded blood pressure probably not representative.

‡ Left sympathectomy on May 20, 1940. Reference values for  $C_{IN}$  and  $C_D$  taken as one-half of bilateral values obtained on May 3, 1940.§ Basal  $T_{MD}$  not available, so equated with value obtained on June 7, 1940. Bilateral sympathectomy in June, 1938.

|| Bilateral sympathectomy in May and June, 1939.

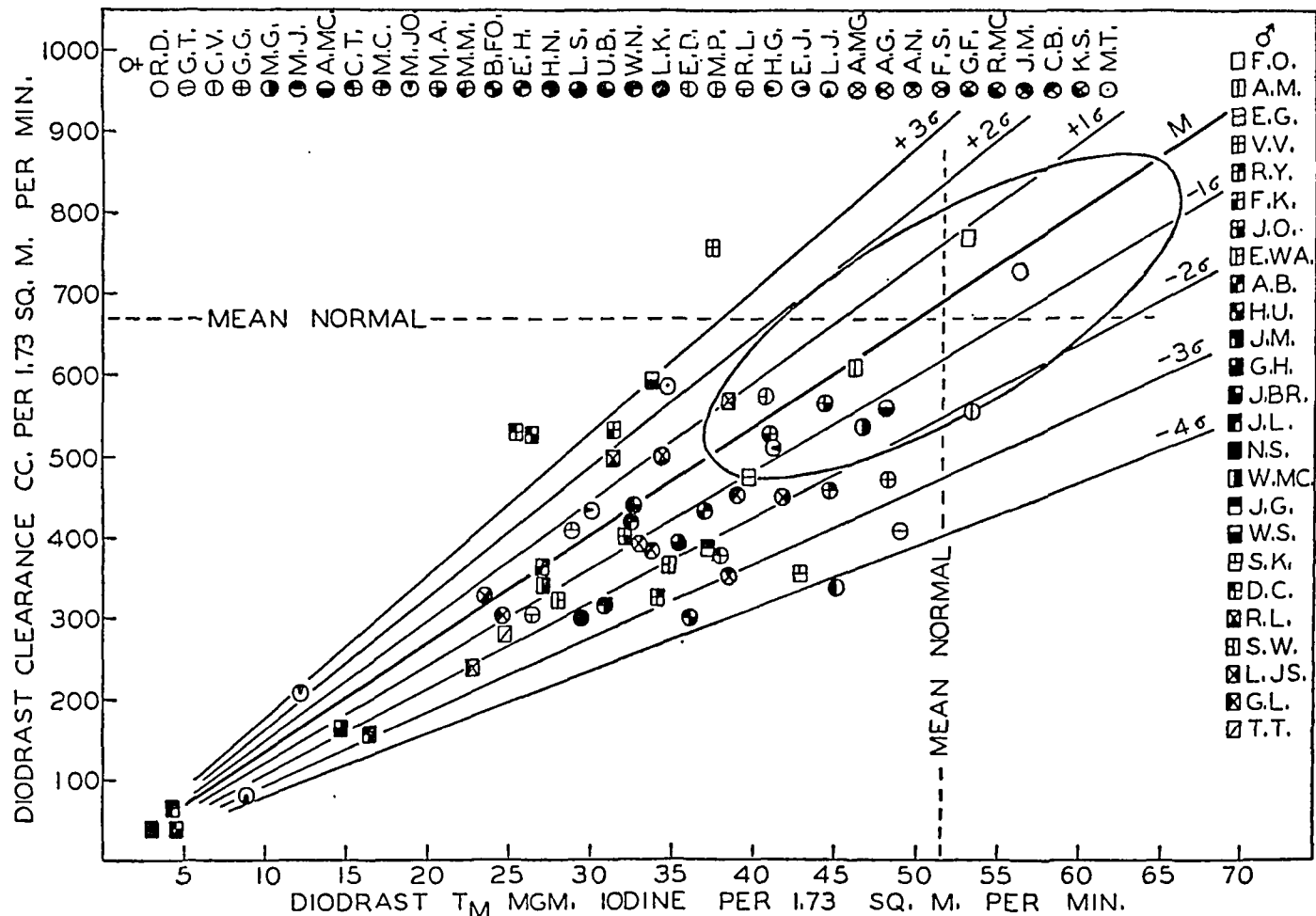


FIG. 1. DIODRAST CLEARANCE (EFFECTIVE RENAL PLASMA FLOW) IN HYPERTENSIVE SUBJECTS, RELATED TO DIODRAST  $T_m$  (TUBULAR EXCRETORY MASS)

The statistical background is based on data from normal subjects (9), the ellipse being calculated to contain 70 per cent of the normal data, and actually containing 72 per cent.

especially since the present group of patients all had well-established hypertensive disease.

With the exception of three subjects (F. O., R. D. and V. V.),  $C_D$  is below the mean normal value (669 cc. per minute) and, like  $Tm_D$ , ranges from slightly subnormal to very low values. A reduction in  $C_D$  may result from either a decreased total renal blood flow or a decreased extraction ratio (see uncleared blood (12)). Since we cannot at present distinguish between these alternative explanations, we cannot say whether the total renal blood flow, *i.e.*, the sum of uncleared plus cleared blood, is decreased in these subjects or not. A reduction in the volume of blood cleared of diodrast implies, however, a reduction in the volume of blood cleared of such endogenous products as the tubules are normally called upon to excrete, and therefore an impairment of an important renal function. With

regard to the reduction in extraction ratio, which probably occurs in these subjects, we have elsewhere (12) emphasized the importance of referring the datum  $C_D$  to  $Tm_D$  by means of the ratio  $C_D/Tm_D$ , which expresses the relative quantity of plasma ( $C_D$ ) cleared by the residual functional tissue,  $Tm_D$ . It is in keeping with the physiological implications of this ratio to speak of increments or decrements beyond the normal or expected limits as a relative hyperemia or ischemia of the residual functional tissue.

As can be seen from data in Figure 1, the ratio  $C_D/Tm_D$  is distributed about the mean value in an uneven manner, 45 out of 60 subjects falling on or below  $M$ . This preponderant distribution below  $M$  suggests that some factor is operating in hypertensive subjects to produce a relative ischemia in the residual functional tubular tissue. This relative ischemia is, we believe, one of the

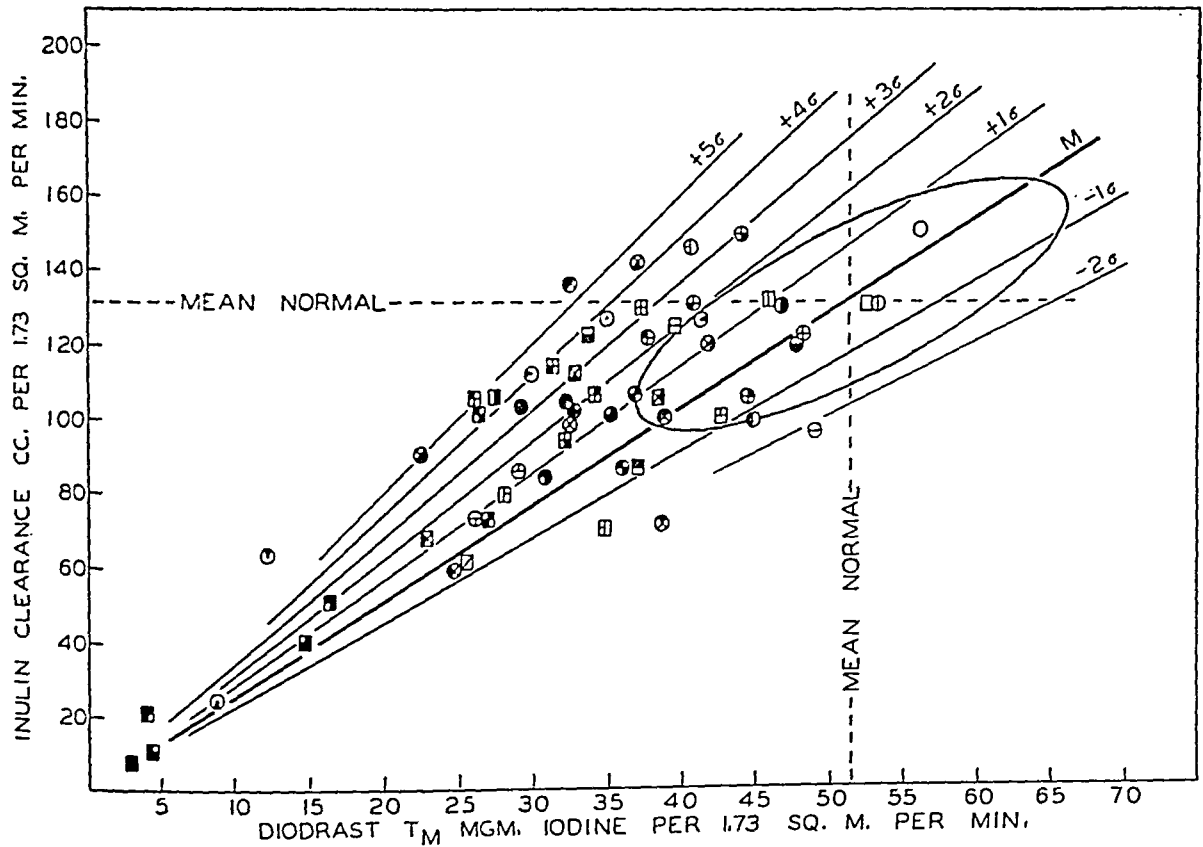


FIG. 2. INULIN CLEARANCE (FILTRATION RATE) IN HYPERTENSIVE SUBJECTS, RELATED TO DIODRAST  $T_m$ . Statistical background as in Figure 1. The ellipse, again calculated to contain 70 per cent of the normal data, actually contains 75 per cent.

major physiological disturbances characteristic of the hypertensive kidney, and will be discussed later in the paper.<sup>4</sup>

## 2. Inulin clearance and diodrast $T_m$

Figure 2 presents the inulin clearance ( $C_{IN}$ ) in relation to  $T_{mD}$ , the mean normal values of  $C_{IN}$  and  $T_{mD}$  and of the ratio  $C_{IN}/T_{mD}$  (the heavy line marked  $M$ ) being taken from the normal data referred to above. Again the ellipse is calculated to contain 70 per cent of the normal observations, and actually contains 75 per cent.

<sup>4</sup> Chesley and Chesley (4) have examined  $C_D$  in eleven hypertensive women and have also observed a decrease in this datum, associated with an increased filtration fraction, as estimated from the urea clearance, in eight of their patients. Since  $C_{IN}$  and  $T_{mD}$  were not determined, it is not possible to subject their data to the interpretive analysis to which our data are subjected in this paper. On the whole, however, their data are in agreement with our present observations.

In all but five subjects (R. D., M. C., R. L., F. S. and K. S.),  $C_{IN}$  is on or below the mean normal value (131 cc. per minute). There is, however, a tendency for  $C_{IN}$  to remain within the lower range of normal values (92 to 131 cc.) until  $T_{mD}$  has been markedly reduced, as shown by the horizontal, leftward displacement of the data.

Reduction in filtration rate might be caused by (a) decrease in blood pressure, (b) increase in the resistance presented by the afferent glomerular arterioles or the preglomerular vascular bed, (c) thickening of the glomerular membranes to such an extent that equilibrium in respect to filtration pressure is not approached as closely, as in the normal kidney, or (d) by obliteration of glomeruli. Decreased blood pressure is clearly ruled out, since all these subjects have a mean pressure above normal; although the importance to be attached to the contributions of (b) and



fraction ( $C_{IN}/C_D$ ), as plotted in Figures 5 and 6. In normal subjects the filtration rate tends to remain constant when the renal blood flow is increased during pyrogenic hyperemia or decreased by adrenalin, a circumstance which indicates that the locus of changing resistance is at the efferent glomerular arteriole (3, 14).<sup>6</sup> Where the filtration rate is constant, the filtration fraction,  $FF$ , must vary inversely as  $C_D$ , *i.e.*,  $FF$  when plotted against  $C_D/Tm_D$  will describe a rectangular hyperbola, as shown in Figures 5 and 6. In these figures the solid curve and its two dotted parallels represent respectively the course of  $FF$ , if  $FF \times C_D/Tm_D = 2.56 \pm 2\sigma$ , the mean normal value of  $C_{IN}/Tm_D$ . The hexagon represents an arbitrary area which contains 95 per cent of the normal data under basal conditions; under adrenalin,  $FF$  rises above the limits of the hexagon, while during pyrogenic hyperemia it falls below the hexagon, though in both instances it remains between the dotted parameters of mean  $C_{IN}/Tm_D \pm 2\sigma$  (see Figure 2 of our previous paper (14)).

The data on hypertensive subjects, as shown in Figures 5 and 6, are divided into two categories: those to the left of the vertical dotted line represent basal observations, and those to the right represent observations made during

hyperemia. (The data on experimentally induced hyperemia will be discussed in the next section. The effects of adrenalin on hypertensive subjects have not been examined.)

The unselected hypertensive series as a whole (basal data, Figure 5) shows a wide scattering of  $FF$  relative to  $C_D$ , though in almost all instances  $FF$  is greater than the mean normal value of 19 per cent, and may reach values as high as those reached under the maximal action of adrenalin in normal subjects. Since the relative ischemia in these hypertensive subjects is correlated with a rise in  $FF$ , we conclude that the ischemia is the result of increased resistance beyond the glomeruli. With no serious consequences if the assumption is not wholly correct, we may suppose that the major increase in resistance in the hypertensive kidney is in the efferent arterioles, rather than located in part in the postglomerular capillary bed. (We recognize that this assumption may have to be abandoned when further information is available on the dynamics of the postglomerular circulation.)

In many instances  $FF$  is higher than could be explained, in terms of the behavior of the normal kidney, on the basis of efferent constriction alone. Such a result is to be expected if, as suggested in Section 2, there exist in some hypertensive subjects impotent nephrons which are supplying glomerular filtrate but not clearing the postglomerular blood of diodrast, or if the filtration rate in some nephrons is increased by elevated glomerular pressure. Consequently, we have deleted from Figure 5 those subjects in Group A, as defined above, who on the basis of a high  $C_{IN}/Tm_D$  ratio were deleted in Figures 3 and 4. The selected group which remains (Group B) in Figure 6 (referring to the basal data only) now shows values of  $FF$  which, though elevated above the mean normal value, nonetheless fall within the normal parameters with respect to  $C_D/Tm_D$ . In short, Group B presents an uncomplicated picture of renal ischemia induced by moderate to severe efferent arteriolar constriction.

#### 4. The induction of renal hyperemia in hypertensive subjects

We have previously reported that certain non-specific pyrogens in adequate doses, accompanied

<sup>6</sup> Lamport (J. Clin. Invest., 1941, 20, 535, 545) has recently offered cogent and constructive criticisms of our quantitative interpretation of afferent and efferent regulation in the glomerular circulation. Lamport's equation defining the filtration rate when variations in resistance are located in the afferent arterioles yields results not greatly differing from our own, and in any case is of less importance in the present problem than is the equation dealing with variations in efferent resistance. Here our equation leads to a constant filtration rate over a wide range of renal blood flow (neglecting subsidiary factors such as viscosity, etc.), while Lamport's equation leads to a reduction in filtration rate as the blood flow is either increased above or reduced below the basal value by pure efferent constriction; *i.e.*, reduction in blood flow with constant filtration rate would, according to Lamport, require some measure of simultaneous afferent dilatation, which, of course, might follow passively in consequence of an increase in glomerular pressure. Without considering here the premises and equations involved, we may record our uncertainty concerning the validity of this particular interpretation, and note that, even accepting Lamport's equation, which requires some afferent dilatation, *efferent constriction* is still indicated as the cause of the relative renal ischemia in group B of our hypertensive subjects.

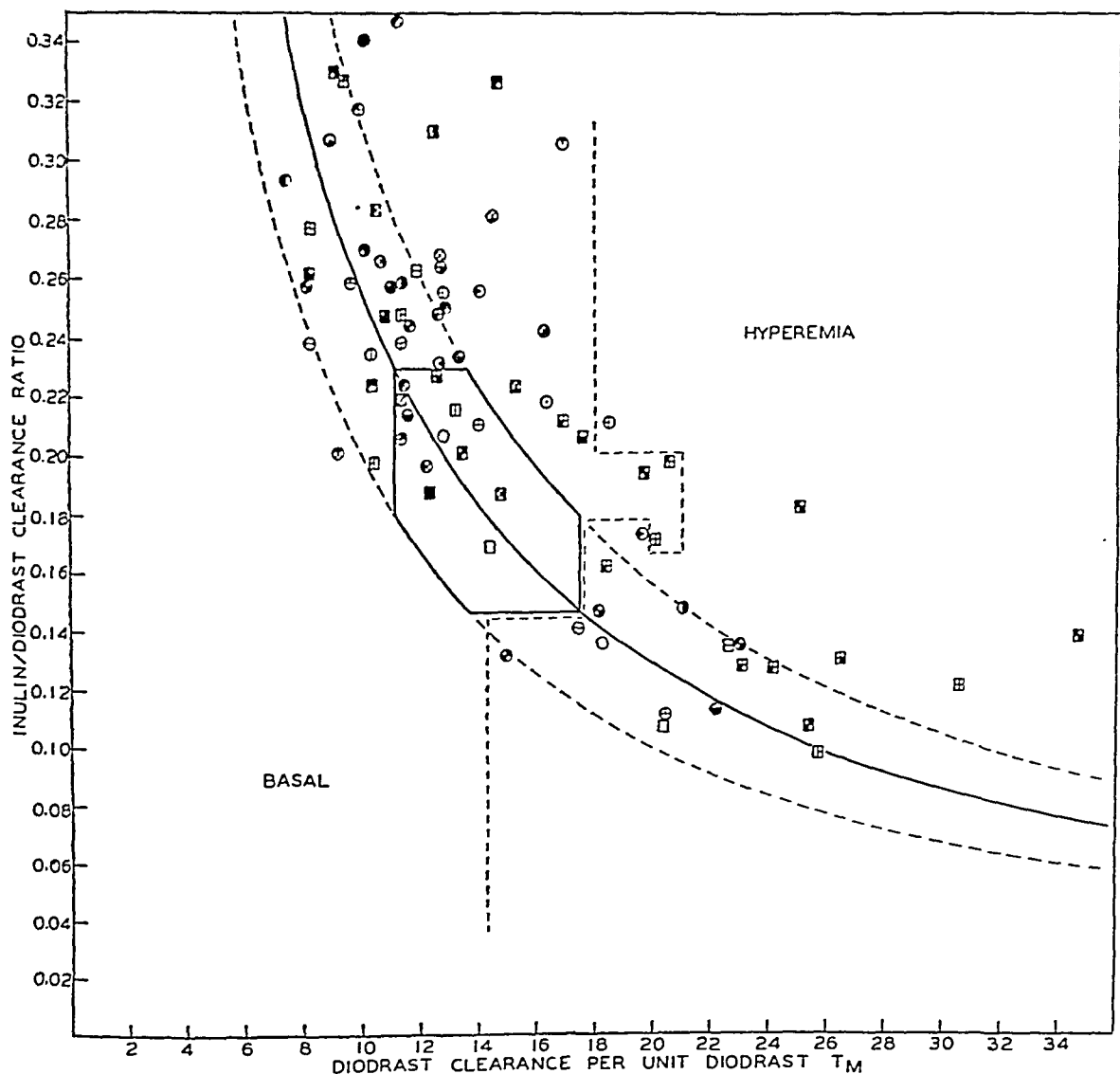


FIG. 5. INULIN/DIODRAST CLEARANCE RATIO (FILTRATION FRACTION) IN HYPERTENSIVE SUBJECTS, RELATED TO THE EFFECTIVE RENAL PLASMA FLOW PER UNIT OF TUBULAR EXCRETORY MASS

The statistical background is again taken from data on normal subjects (14), the hexagon containing 95 per cent of the normal basal data. Basal observations on hypertensive subjects are shown to the left of the vertical dotted line, observations during induced renal hyperemia to the right.

by amidopyrine to reduce or eliminate the general autonomic disturbances of the pyrogenic reaction, produce in normal subjects a moderate to very marked renal hyperemia by efferent dilatation (3, 13, 14). Since this is the only method known to us to induce renal hyperemia, we have utilized it in the examination of the vascular responses of the kidney in hypertensive subjects. The pyrogen used here was a sample of highly

pyrogenic inulin which had been used extensively for this same purpose in normal subjects.<sup>7</sup>

<sup>7</sup> The physiological mechanism of this hyperemia is unknown. That it does not involve inhibition of renal vasoconstrictor fibers is suggested indirectly by the fact that in normal subjects the renal vasoconstrictor fibers are basally inactive (17) and in hypertensive subjects renal denervation does not consistently produce renal hyperemia (1, 2, 7). That it does not involve activation of sympathetic vasodilator pathways is suggested by our observa-

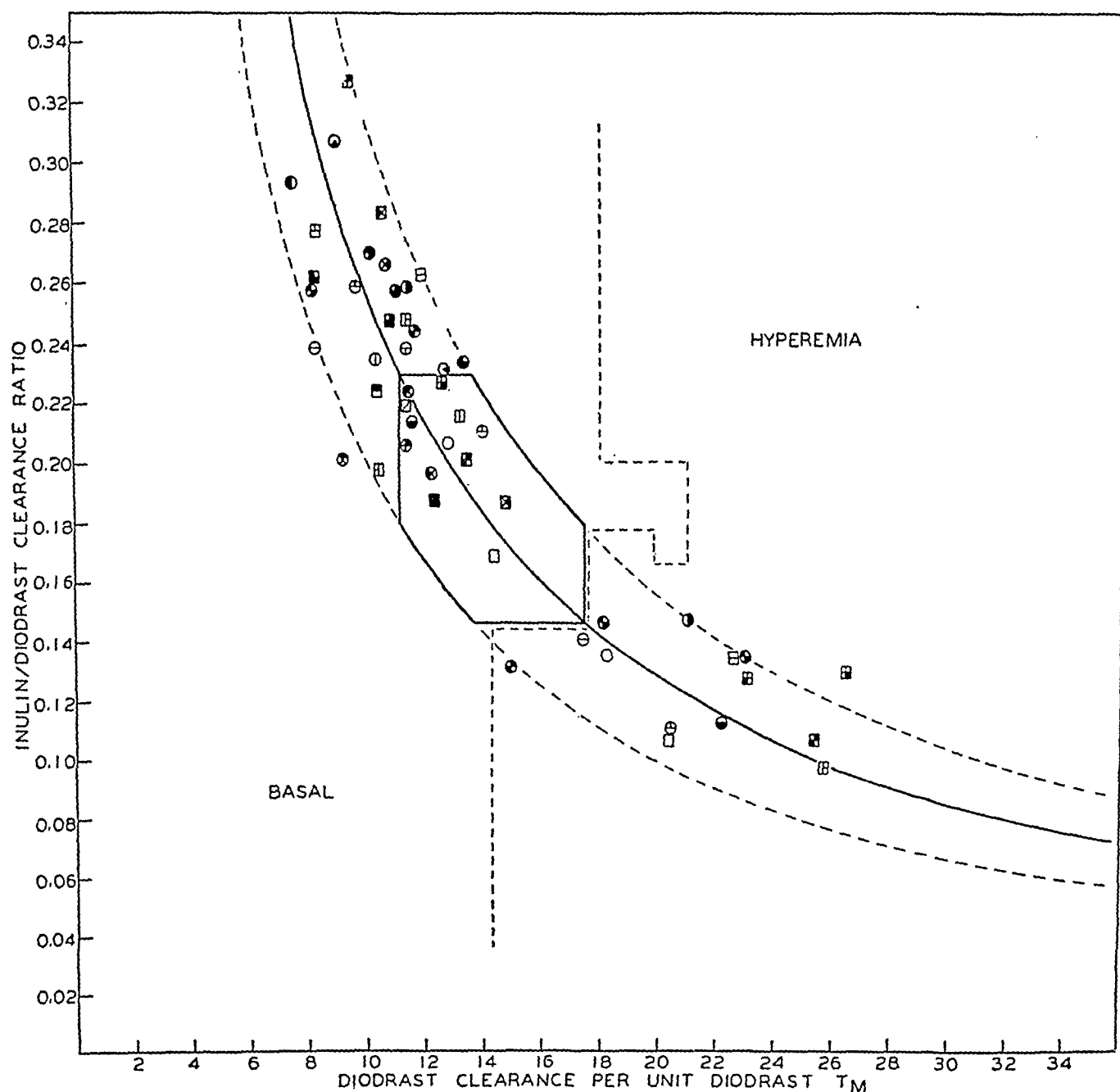


FIG. 6. FIGURE 5 WITH THE SAME SUBJECTS DELETED AS IN FIGURES 3 AND 4

Our method has been to administer 0.6 gram of amidopyrine every four hours, beginning at tions that a fair degree of pyrogenic hyperemia was observed in three sympathectomized patients, E. H., W. Mc. and B. Fo., as recorded in Table III. But since vasodilator fibers may conceivably reach the kidneys by other than the thoracico-lumbar sympathetics, this does not conclusively demonstrate the humoral nature of the renal reaction.

Until more is learned about the mechanism of the hyperemic reaction itself, it is useless to speculate why pyrogen should produce hyperemia in hypertensive subjects where ischemia is presumably referable to a humoral pressor agent.

1 p.m. on the afternoon before the test. At 8 a.m. on the morning of the test 100 to 200 mgm. of the pyrogenic inulin, dissolved in saline and sterilized by a single boiling of ten minutes, were given intravenously. Clearance collections were begun about 9:30 a.m., three ten- to fifteen-minute periods being taken for  $C_D$  and five periods for  $Tm_D$ . The results of these observations are given in Table III. In compiling this table we have chosen as a standard of reference for the basal state the last preceding observation made under basal conditions.

In comparing the hyperemic with the basal data, it will be observed that with few exceptions the change in filtration rate is negative, but so slight as to be scarcely significant. (The marked fall in filtration rate in H. U. on May 1, 1940, was associated with a severe reaction involving a period of acute peripheral circulatory failure.)

Only two subjects out of twenty (J. M. and H. G.) failed to show an increase in  $C_D$  exceeding 25 per cent of the basal value; since these subjects had marked retinopathy and marked proteinuria (Table I), as well as the lowest values of  $Tm_D$  of all the subjects examined with pyrogen, and both died of the renal disease within a month of the observation, we infer that their failure to respond is related to the circumstance that they were in the advanced stage of the disease. It is difficult to evaluate the degree of hyperemia in the other subjects quantitatively. Normal subjects vary considerably in sensitivity to pyrogen and this is doubtless true of hypertensive subjects. In the latter,  $C_D/Tm_D$  during hyperemia ranges from 17.5 to 34.7 and averages 22.8, figures which are comparable with those shown in Figure 2 of our previous paper (14) on a smaller series of normals in which hyperemia was induced; consequently, we feel that the magnitude of hyperemia obtained in the hypertensive subjects is equal to that obtained in normals.

With regard to changes in  $FF$  during hyperemia, we refer again to Figures 5 and 6, where the hyperemic data are shown to the right of the vertical dotted line. Where  $FF$  is abnormally high under basal conditions (*i.e.*, lying outside the normal parameters, as in subjects H. U., V. V. and L. J.), it retains this anomalous relation during hyperemia, a result consonant with the interpretation that in these subjects  $C_{IN}$  is high, relative to  $Tm_D$ , because of the existence of impotent tubules and/or elevated glomerular pressure. Deletion of those subjects (Group A) who were removed for this reason from Figures 3 and 4 leaves a group (B) in which  $FF$  falls within the normal parameters basally, and remains there during hyperemia (Figure 6).

Were the hyperemia attributable to dilatation of the afferent glomerular arterioles, we would expect both  $C_{IN}$  and  $FF$  to increase markedly; our failure to observe this effect in a single instance reinforces our previous conclusion that

the ischemia of the hypertensive kidney is a consequence primarily of increased tone of the efferent glomerular arterioles.

### 5. Humoral origin of increased efferent tone

That the increased efferent tonus in essential hypertension is not caused by a structural, obstructive lesion is, we think, demonstrated by our observations that the renal blood flow can be increased and the filtration fraction lowered by agents which produce these effects in the normal kidney.

That the efferent hypertonus is not of neurogenic origin is indicated by the fact that various operations intended to denervate the kidneys fail in general to increase the renal blood flow, or if the mean blood pressure is not substantially reduced, to lower the filtration fraction (1, 2, 7). The single subject (E. H.) examined by us before and after unilateral sympathectomy<sup>8</sup> showed no difference in  $C_D$  or  $FF$  between the operated and unoperated side during the development of pyrogenic hyperemia. Nor are these values significantly different from other hypertensive subjects in the two subjects (W. Mc. and B. Fo.) who were examined postoperatively. Admitting the difficulty of establishing complete denervation of the human kidney, and recognizing that the above evidence does not logically exclude a local or myogenic origin for the increased efferent tone, we tentatively accept the humoral theory.

Page and Helmer (11) have prepared a crystalline pressor substance (angiotonin) by the reaction between renin and plasma, and Corcoran, Kohlstaedt and Page (5, 6) have shown that this substance produces renal ischemia by efferent arteriolar constriction in dog and man. Through the courtesy of Doctors Page and Helmer, who have supplied us with some of this substance, we have been able to confirm their observation in normal man. These investigators have suggested that angiotonin is responsible for the increased efferent tonus in essential hypertension. The present study throws no light on this question, but from our experience with pressor amines such as adrenalin (3), neosynephrin, cobefrin, tyramine, paredrinol, etc.

<sup>8</sup> This subject was sympathectomized by Dr. Norman Freeman.

(unpublished data), it seems probable to us that a variety of substances will constrict the efferent arterioles and, until further evidence is available, we hesitate to accept the view that angiotonin or any other one substance is the specific humoral agent involved in hypertensive disease in man.

6. Changes in diodrast  $Tm$  during hyperemia

Diodrast  $Tm$ , conceived to be an index of the total active tubular (excretory) tissue (15), has special significance in the hyperemic studies inasmuch as tubules rendered inactive by ischemia under basal conditions might become active, in consequence of opening of vascular channels, during hyperemia. In this connection it must be noted that the contribution of a particular tubule to  $Tm_D$  will be maximal only so long as the plasma flow to the tubule is adequate, at the existing diodrast plasma concentration,  $P_D$ , to effect saturation. Consequently, the use of  $Tm_D$  to detect inactive (ischemic) tubular tissue is contingent upon  $P_D$  in the sense that the higher  $P_D$  is, the lower must be the blood flow to the ischemic tubular tissue before the latter will cease to contribute to  $Tm_D$ . In terms of the overall function of the two kidneys, we may calculate the load of diodrast carried to the tubules as the product of the plasma flow times  $P_D$ , minus the quantity of diodrast excreted through the glomeruli; if we take the plasma flow equal to  $C_D$ , as observed immediately before  $Tm_D$  measurement, then

$$\text{tubular load} = P_D(C_D - FWC_{IN}),$$

where  $FW$  is the fraction of free or ultrafiltrable diodrast at the plasma level,  $P_D$ , actually present during  $Tm_D$  determination. It is convenient to refer the load to  $Tm_D$ , as in column 10 of Table III.

In the observations made here, the load/ $Tm_D$  ratio has with very few exceptions been greater during hyperemia than during basal conditions (in part because of the increase in  $C_D$ ); this circumstance would operate, quite apart from any increase in blood flow, to saturate any tubules which might have been unsaturated in consequence of even severe ischemia during the basal state.

In three subjects (C. V., E. H. and J. M.)

$Tm_D$  decreased during hyperemia by more than 10 per cent; in C. V. there was a distinct fall in mean blood pressure during  $Tm_D$  measurement, which may have reduced perfusion to some parts of the kidney. J. M. was examined during the accelerated phase of the disease and died within one month after the last observation; at necropsy the kidney showed multiple abscesses and necrotizing arteriolar lesions. The observations on E. H. appear to be technically satisfactory. In seven subjects  $Tm_D$  increased by more than 10 per cent, the largest increase being +33 per cent (H. G.); while in ten subjects there was no significant change (less than 10 per cent) in  $Tm_D$  during hyperemia, despite changes in  $C_D$  ranging up to 88 per cent.

In interpreting the fact that  $Tm_D$  fails to increase in so many subjects during hyperemia, it may be noted that, although the pyrogenic reaction appears to involve a humoral vasodilatory agent, so far as the efferent arterioles of the kidney are concerned, this is probably not the only vasomotor disturbance. Considered alone, it is difficult to predict whether efferent arteriolar dilatation would necessarily restore perfusion in portions of the renal parenchyma to which the blood flow had been reduced by functional constriction or obstructive lesions in the preglomerular arterial bed; and when combined with a decrease in mean blood pressure and possibly with changes in the status of the preglomerular arterioles, it is not on the whole surprising that a variety of results may be obtained, even in the same individual, in consequence of the experimental induction of hyperemia.

What appears to us to be the important fact is that in some subjects a significant increase in  $Tm_D$  has been observed during hyperemia, demonstrating that substantial portions of renal parenchyma capable of excreting diodrast were not available to perfusion under basal conditions.

It is not to be concluded, however, that the local ischemia is necessarily a direct consequence of the increased tone of the efferent glomerular arterioles. We have, in general, been unable to produce any decrease in  $Tm_D$  with adrenalin in normal subjects, and it seems improbable to us at the present time that efferent constriction in the normal kidney could completely obstruct the glomerular, and hence the postglomerular, circu-

lation. In view of the well-recognized fact that the arteriolar lesions in the hypertensive kidney are most evident in the preglomerular and afferent arterioles, and that these lesions are in many instances of such a nature as to narrow or obstruct the lumen, we cannot avoid the inference that it is in these lesions that we must at least in part seek the basis of occluded renal parenchyma.

### 7. Changes in diodrast $Tm$

With regard to the constancy of  $Tm_D$ , in studies to be reported elsewhere we have been unable in normal subjects to produce significant changes in this value by the induction of hyperemia or by the administration of adrenalin or caffeine. In ten of the hypertensive subjects listed in Table II we have obtained fair agreement in  $Tm_D$  on repeated examination (G. H., E. D., L. K., R. Y., M. Mc., G. G., M. A., F. K., L. J. and H. U.), in some of these over a period of two to three years. In two subjects, however (M. G. and M. Jo.), there have been notable but unexplained changes.

Between December 20, 1937, and February 9, 1938, when the patient M. G. had been continuously in the hospital, her  $Tm_D$  fell from 53.2 to 41.3. This latter value was obtained again after a three-year period. It is, of course, possible that this represents a rapid progression of renal disease, but this interpretation is rendered less certain by our observations on M. Jo.

In M. Jo.  $Tm_D$  decreased from 41.3 to 29.8 between March 21, 1938, and May 1, 1939, only to rise again to 35.9 by April 4, 1941. Even more striking is the increase from 35.9 to 45.1 on hospitalization from April 4 to May 7, 1941. We have earlier in the paper given reasons for believing that there is a progressive reduction in  $Tm_D$  during the course of hypertensive disease and our experience with M. Jo. suggests that in some instances there may occur marked transient fluctuations in this value without apparent clinical changes. Such fluctuations might be caused by extreme focal ischemia of tubular tissue which excluded it from perfusion during  $Tm_D$  measurement, or by intrinsic loss of capacity on the part of the tubules to excrete diodrast with subsequent regeneration or compensatory hypertrophy in other nephrons.

With regard to changes in  $Tm_D$ , it is pertinent that White, Heinbecker and Rolf (18) have demonstrated that in the dog  $Tm_D$  is substantially reduced by anterior hypophysectomy, while Winternitz and his collaborators (19) have shown that *in vitro* autolysates of renal tissue contain cytotoxic agents which attack arteriolar and other tissue throughout the body, and such agents, if present in hypertensive disease, might also be injurious to tubular function. Our data indicate that the progressive loss of tubular function, as measured by  $Tm_D$ , is characteristic of the course of hypertensive disease, but whether or not the same factors produce both temporary and progressive changes in tubular function is unknown.

### SUMMARY AND CONCLUSIONS

The filtration rate ( $C_{IN}$ ), diodrast clearance ( $C_D$ ) and the maximal rate of tubular excretion of diodrast ( $Tm_D$ ) have been examined in sixty subjects with essential hypertension. Comparison of the data with those previously reported for the normal kidney reveals the following facts:

An extreme reduction in  $Tm_D$  occurs in advanced states of the disease, and for the entire series of sixty subjects  $Tm_D$  is below or in the lower normal range; these facts lead us to infer that the disease is characterized by a progressive impairment of tubular function which proceeds at varying pace in different subjects.

In some individuals impairment of tubular function appears to outrun impairment of glomerular function (formation of impotent tubules), so that the filtration rate remains within the limits of normal variation when  $Tm_D$  has been substantially reduced. In the nature of the renal circulation, elevation of the mean systemic blood pressure or the formation of impotent tubules may increase the quantity of diodrast-containing blood perfusing the residual functional tissue. We believe that in either case the anomalous condition will be revealed by the presence of a high filtration rate per unit of functional tubular tissue.

Deleting such anomalous instances, the effective blood flow per unit of functional tubular tissue, or the ratio  $C_D/Tm_D$ , in the remaining subjects, ranges downward from the mean normal

to highly subnormal values, indicating relative renal ischemia. Since this ischemia is associated with an elevation of the filtration fraction, it is attributed to increased tone of the efferent glomerular arterioles. On the available evidence, this increased efferent tone may in turn be attributed to the presence of one or more pressor substances in the blood. The increased efferent tone is functionally reversible, in that renal hyperemia, associated with a fall in filtration fraction (efferent dilatation), follows the administration of suitable doses of pyrogen, as in normal subjects. The absolute values of  $C_D/Tm_D$  in hypertensive subjects during hyperemia are of the same order of magnitude as in normals during the hyperemic reaction.

In most hypertensive subjects,  $Tm_D$  has been reasonably constant over a considerable period, and has not increased during pyrogenic hyperemia. In some subjects, however,  $Tm_D$  was increased during hyperemia, indicating that in these and perhaps in other subjects substantial quantities of tubular tissue may be ischemic under basal conditions. Spontaneous changes in  $Tm_D$  have been observed which may reflect changes in the quantity of tubular tissue available to perfusion or trophic changes in the excretory tissue itself.

In brief, the functional picture presented by the hypertensive kidney is consonant with the theory that there is present in the blood in hypertensive disease one or more pressor substances which produce a reversible renal ischemia by constriction of the efferent glomerular arterioles. In addition, there is profound impairment and ultimate destruction of tubular function. Which of these precedes the other is as yet undetermined.

There is no evidence in the present investigation to warrant the conclusion that renal ischemia is the primary cause of essential hypertension. The renal ischemia demonstrated here, which has its origin in increased tone of the efferent glomerular arterioles, appears to be one of the sequelae of the hypertensive process. We may place on record our belief that primary renal ischemia in man can, under proper quantitative circumstances, initiate a hypertensive process, but whether or not the secondary ischemia, associated with efferent hypertonus, which is present in hypertensive subjects generally, contributes

to the progress of the disease cannot be answered from this study. Alternatively, the possibility cannot yet be excluded that the appearance of pressor and cytotoxic substances in the blood follows a metabolic disorder in the kidney or in other organs, and is wholly independent of renal ischemia.

The analysts in this work have been Helen A. Keigher, Katharine S. Tilson, Frances E. Marx, Betty J. Crawford and Martha J. Barrett. We are indebted to them, and to Nurse Ann S. Rivoire and Nurse Helen R. McGuire for their unflinching cooperation.

The clearance technique and analytical methods used in this study were identical with those described in a previous paper (9). The inulin for the most part was ampouled material obtained from the U. S. Standard Products Company, Madison, Wisconsin; the diodrast, which was supplied in part by courtesy, was obtained from Winthrop Chemical Company, New York; and the saline was prepared by the Sterisol Ampoule Company (Schering and Glatz, Inc.), New York.

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# EFFECTIVE RENAL BLOOD FLOW IN THE SEPARATE KIDNEYS OF SUBJECTS WITH ESSENTIAL HYPERTENSION<sup>1</sup>

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Unilateral renal disease in man has been widely accepted as a primary cause of arterial hypertension. The assumption is made that there has been obstruction to the blood flow in the diseased kidney and that the resulting ischemic renal tissue is directly responsible for the abnormal elevation of the blood pressure. If such were the case, it seemed to us that it could be demonstrated by the examination of the separate kidneys, and this report concerns the application of the clearance methods to this problem.

## 1. Application of the clearance method to unilateral studies

The possible difficulties<sup>2</sup> which present themselves when applying the clearance technique to unilateral measurements lie in the failure to obtain a total collection of urine by means of ureteral catheters, and the excitation of reflex renal vasoconstriction by trauma to the bladder and ureters or physical and mental discomfort associated with prolonged catheterization.

On the question of complete collection, we find that if a Number 8 French, flute-tipped, radio-opaque, silk catheter with six eyes is passed only 12 cm. up the ureter, *i.e.*, into the portion of the ureter having the narrowest lumen, extra-catheter flow is usually avoided. The occurrence and extent of leakage around the catheter can be detected by addition of phenol red to the infusion fluid, and by inserting a third catheter in the bladder; the bladder can thus be rinsed at intervals, when leakage will be revealed by the appearance of dye in the bladder urine. Where it is possible to identify the side on which leakage

occurs, analysis of bladder urine may be used to correct the data on the appropriate kidney.

With regard to reflex vasoconstriction, we believe that it occurred once in the forty-five subjects whom we have examined. In this instance it was concluded that there was vasoconstriction of the afferent glomerular arterioles, manifesting itself by a marked decrease in renal blood flow, filtration rate (constant filtration fraction) and urine volume; the spasm lasted for about twenty minutes with a gradual return to normal function at the end of one hour. Otherwise, the values observed by us in unilateral studies are comparable to those obtained in bilateral studies. Again, in ten patients who had both unilateral and bilateral tests, satisfactory agreement was found in all the values obtained by both methods.

## 2. Selection of subjects

Patients with hypertension were selected at random from the Nephritis and Hypertension Clinic of the New York University College Clinic and from the wards of the Third (New York University) Medical Division of Bellevue Hospital. There were seventeen female and four male subjects. These subjects were considered to have essential hypertension and were representative of the entire clinic population with this diagnosis. No attempt was made to choose or exclude those patients who gave evidence of urinary tract disease. It is important to note that in this random selection no instance of advanced destructive unilateral renal disease was encountered.

In addition to the twenty-one subjects here reported, hypertensive patients who had undergone splanchnicectomy, renal-omentopexy and nephropexy were also studied by the same technique and will be reported elsewhere.

Observations were also made on five normal individuals for the purpose of establishing the technique of the procedure. These normal subjects were convalescent, volunteer ward patients, in whom no evidence suggestive of renal or hypertensive disease was obtained.

## 3. Procedure

To reduce the urine flow and prevent leakage, the patients were taken off fluids for a sixteen-hour interval preceding the period of observation. They were examined

<sup>1</sup> Aided by a grant from the Commonwealth Fund.

<sup>2</sup> Accurate determination of the rate of urine flow might not be obtainable from a large hydronephrotic kidney, and the clearance method could be employed accurately only if the plasma concentration of diodrast and inulin were kept constant, and if no urine accumulated in the dilated pelvis.

without breakfast, the examination being made with the patient in the supine position. One-half hour before cystoscopy the majority of the patients received 0.20 gram of pentobarbital by mouth. It is believed that this sedation did not affect the measurements since repetition of the test without sedation in the same patient yielded comparable data. Following routine cystoscopic examination, Number 8 ureteral catheters were inserted about 12 cm. up the ureters. In those instances where we failed to introduce a Number 8 F., Number 7 F. or Number 6 F. catheters were used. The cystoscope was then removed, except in occasional instances when it was allowed to remain in position during the period of observation. A multi-eyed urethral catheter was then inserted into the

bladder. An infusion of phenol red, diodrast and inulin was given throughout the period of observation. Following the discard period, either three urine collection periods or a single long urine collection period of about twenty-five minutes were taken and the rate of glomerular filtration and effective renal blood flow measurements were made in this time interval. In those patients in whom the measurement of the tubular excretory mass was to be made, a second series or a single urine collection period was taken after a twenty-minute discard period. For detailed data concerning the preparation of the infusion fluid, the chemical analytical methods, etc., the reader is referred to previous papers (1, 2).

Immediately following the clearance studies, retrograde

TABLE I  
*Observations on the separate kidneys of hypertensive subjects*  
(Columns 3, 4, 5, 6, 7, and 8 corrected to 1.73 sq. m., Column 8 corrected to 98.5° F.)

Subject	Date		Plasma clearances				Effective blood flow	Filtration fraction	Diodrast <i>T</i> <sub>m</sub>	Diodrast clearance	Inulin clearance	Plasma flow Δ*
				Inulin	Phenol red	Diodrast				Diodrast <i>T</i> <sub>m</sub>	Diodrast <i>T</i> <sub>m</sub>	
			<i>kidney</i>	<i>cc. per minute</i>	<i>cc. per minute</i>	<i>cc. per minute</i>	<i>cc. per minute</i>	<i>per cent</i>	<i>mgm. iodine per minute</i>			<i>per cent</i>
HYPERTENSIVE SUBJECTS												
M. J.	May	20, 1940	R	73.3	185	282	454	26.0	22.6	12.4	3.24	— 6.0
			L	65.9	184	254	408	25.9	24.2	10.5	2.72	— 15.3
A. Mc.	April	22, 1940	R	46.8	122	154	249	30.3	20.1	7.7	2.33	— 48.7
			L	51.6	139	182	295	28.3	25.0	7.3	2.06	— 39.3
M. C.	March	29, 1940	R	66.1	146	250	370	26.5	20.9	12.0	3.16	— 16.7
			L	82.6	198	315	465	26.2	23.4	13.4	3.53	+ 5.0
L. Js.	December	19, 1940	R	57.6		294	550	19.6	19.4	15.2	2.97	— 14.5
			L	56.4		274	512	20.6	20.7	13.2	2.73	— 20.3
M. M.	April	8, 1940	R	61.1	150	194	377	31.4	18.9	10.3	3.23	— 35.3
			L	59.7	147	185	358	32.2	19.0	9.8	3.14	— 38.3
A. G.	October	4, 1940	R	35.7		176	284	20.3	18.9	9.3	1.89	— 41.3
			L	36.2		180	295	20.1	19.7	9.1	1.84	— 46.2
A. N.	November	4, 1940	R	59.2		225	357	26.3	21.8	10.3	2.71	— 25.0
			L	60.5		227	360	26.6	20.1	11.3	3.01	— 24.3
R. Mc.	November	18, 1940	R	45.1		200	330	22.5	16.7	12.0	2.70	— 33.3
			L	49.7		216	357	23.0	20.2	10.7	2.41	— 28.0
F. S.	November	14, 1940	R	69.5		243	402	28.7	17.4	14.0	4.00	— 19.0
			L	72.9		261	432	28.2	17.0	15.4	4.28	— 13.0
K. S.	May	12, 1941	R	59.6		166	281	36.0	13.1	12.6	4.50	— 50.4
			L	76.3		222	375	34.0	20.4	10.9	3.70	— 33.7
L. S.	April	15, 1940	R	49.4	121	211	398	23.5	15.4	13.7	3.20	— 29.7
			L	52.2	124	213	401	24.5	17.4	12.2	3.00	— 29.0
A. Mg.	September	27, 1940	R	56.3†		220	392	25.6	14.8	14.9	3.80	— 26.6
			L	56.0†		199	354	28.1	17.8	11.2	3.10	— 33.3
F. K.	May	27, 1940	R	63.0	158	303	564	20.8	16.2	18.7	3.88	— 11.9
			L	68.0	171	330	615	20.6	15.2	21.7	4.47	— 4.1

TABLE I—Continued

Subject	Date	Plasma clearances				Effective blood flow	Filtration fraction	Diodrast $T_m$	Diodrast clearance	Inulin clearance	Plasma flow $\Delta^*$
			Inulin	Phenol red	Diodrast				Diodrast $T_m$	Diodrast $T_m$	
		kidney	cc. per minute	cc. per minute	cc. per minute	cc. per minute	per cent	mgm. iodine per minute			per cent
HYPERTENSIVE SUBJECTS—Continued											
E. D.	June 24, 1940	R	53.8		220	331	24.3	15.8	13.9	3.39	−26.7
		L	48.4		205	308	23.3	14.8	13.8	3.27	−31.6
E. Wa.	April 1, 1940	R	40.2		162	270	24.9	14.9	10.8	2.69	−52.9
		L	40.8		146	243	27.9	14.7	9.9	2.77	−57.8
G. F.	November 25, 1940	R	45.9		225	384	20.4				−25.0
		L	40.2		208	355	19.3				−30.8
C. B.	December 29, 1939	R	52.9	135		381†	23.5				−25.0
		L	50.2	123		348†	24.5				−31.6
R. B.	January 22, 1940	R	51.4	123		348†	25.0				−31.6
		L	51.9	123		348†	25.3				−31.6
S. R.	May 10, 1940	R	58.2		187	328	32.1				−37.6
		L	55.6		173	304	31.1				−42.3
J. C.	January 15, 1940	R	45.6	114		322†	24.0				−36.7
		L	40.4	104		294†	23.3				−42.3
S. K.	October 7, 1940	R	46.3		164	280	28.4				−52.3
		L	53.3		194	332	27.5				−43.6
NORMAL SUBJECTS											
E. H.	November 20, 1939	R		205		610§					+ 7.5
		L		221		658§					+15.8
S. P.	February 26, 1940	R	60	162		509§	21.0				−14.9
		L	70	194		610§	20.5				+ 1.8
D. P.	March 4, 1940	R	57.6	173		503§	18.9				− 9.2
		L	64.9	189		554§	19.5				− 0.9
C. J.	March 20, 1940	R	55.2	183	297	457	18.6				−11.3
		L	51.8	178	281	433	18.4				−16.1
W. J.	March 25, 1940	R	75	237	366	631	20.5	31	11.8	2.42	+ 9.5
		L	67.8	234	360	621	18.8	30.3	11.9	2.24	+ 7.6

\* Variation from one-half the mean value for effective plasma flow in normal men and women.

† Uncertain plasma inulin.

‡  $C_{PR}/C_D$  assumed as 0.60.

§  $C_{PR}/C_D$  assumed as 0.57

pyelograms were made, the results of which will be reported at another time. Blood pressure and body temperature were also determined at the time of examination.

In ten instances out of forty-five a reaction followed catheterization, consisting of fever, costovertebral pain, hematuria and dysuria. These symptoms persisted for about twenty-four hours.

#### 4. Filtration rate, effective blood flow and tubular excretory mass

The data obtained on five normal subjects studied with the unilateral clearance technique

are given in Table I. The combined right and left renal blood flow, filtration rate and diodrast  $T_m$  ( $T_{mD}$ ) measurements obtained in this group agree satisfactorily with those obtained in thirty-five normal subjects observed using the bilateral technique (2). Function is fairly equally divided between the two kidneys.

The data obtained on twenty-one patients with hypertension are given in Table I.  $T_{mD}$  was measured in only fifteen subjects, and the data on the remaining six patients have not been

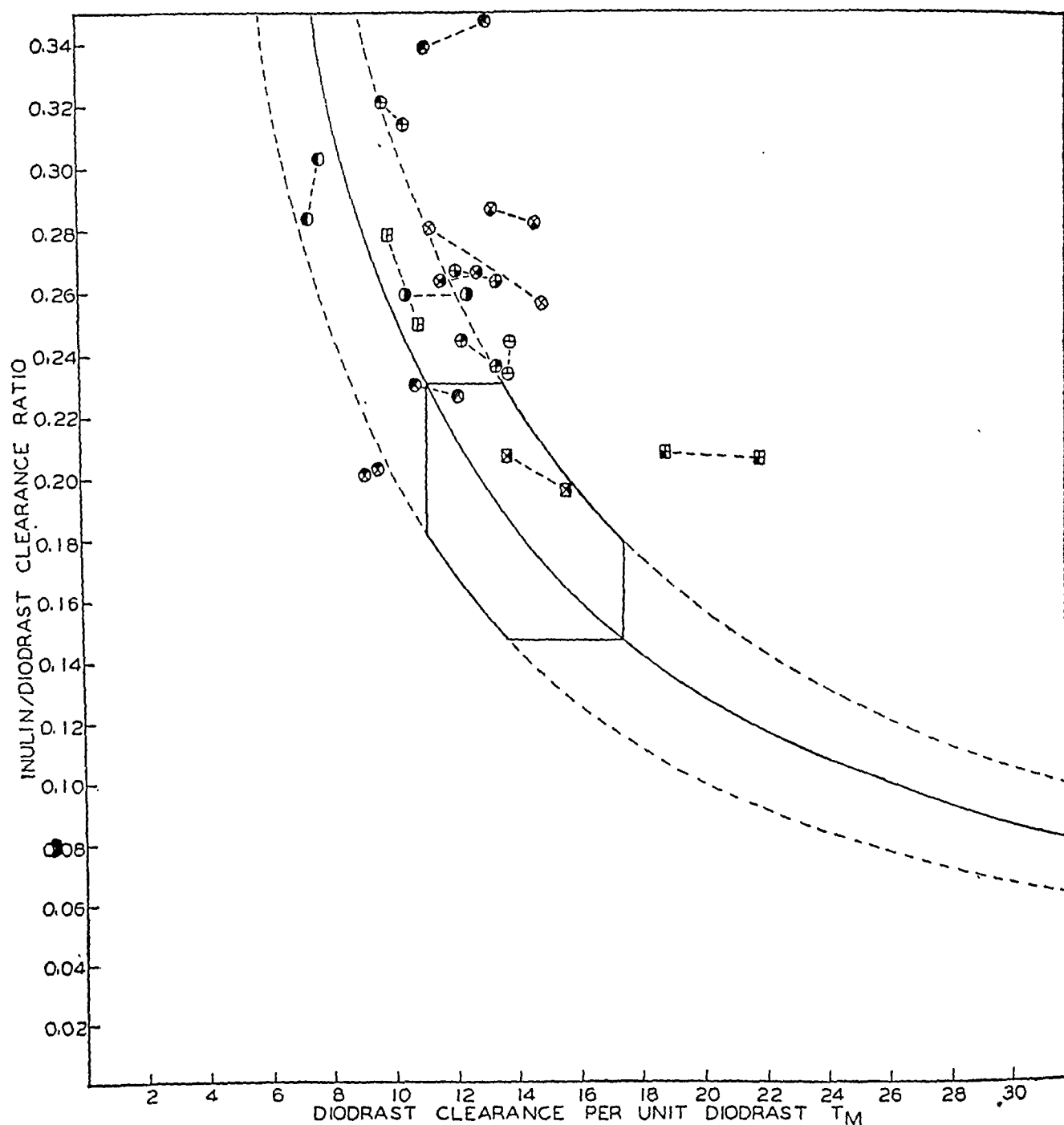


FIG. 3. INULIN/DIODRAST CLEARANCE RATIO (FILTRATION FRACTION) IN THE SEPARATE KIDNEYS OF HYPERTENSIVE SUBJECTS, RELATED TO EFFECTIVE RENAL PLASMA FLOW PER UNIT OF TUBULAR EXCRETORY MASS

Statistical background as in Figure 5 of preceding paper (3).

with respect to  $C_D$ ,  $Tm_D$  and  $C_{IN}/Tm_D$ . In the six hypertensive subjects in whom only  $C_{IN}$  and  $C_D$  were measured, the two kidneys shared equally in a marked decrease in  $C_D$  and a more moderate decrease in  $C_{IN}$ ,  $C_{IN}/C_D$  being invariably above the mean normal value. Although one cannot interpret the data in these six subjects with the same confidence as in the group

in which  $Tm_D$  was measured, the fact that  $C_D$  and  $C_{IN}$  were reduced equally in the two kidneys indicates that in these subjects also unilateral renal ischemia is not present.

#### CONCLUSION

The clearance method has been applied to the measurement of the renal blood flow, the filtra-

tion rate and the tubular excretory mass in the separate kidneys of patients with essential hypertension. The results of these observations indicate that the destruction of tubular tissue progresses equally on the two sides in hypertensive disease and that the functional disturbance in respect to blood flow and filtration rate is shared equally by the two kidneys.

In no instance in the twenty-one hypertensive subjects picked at random is there any indication of a unilateral ischemic kidney. If it is predicated that renal ischemia is the primary causal factor in all essential hypertension, it would be expected that unilateral impairment of renal function would be observed more frequently than

bilateral impairment. The absence of unilateral impairment in these subjects argues against the above premise.

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# AN EPIDEMIC OF INFLUENZA. RESULTS OF PROPHYLACTIC INOCULATION OF A COMPLEX INFLUENZA A-DISTEMPER VACCINE<sup>1</sup>

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Investigations in recent years have demonstrated that clinical influenza may be caused by at least two serologically specific filtrable viruses (1 to 6). It is probable that other etiologic agents will be discovered. The clinical characteristics of influenza caused by different agents are exceedingly uniform and no accurate method of distinguishing them by clinical means has been found (6 to 10). The etiologic diagnosis of influenza must at present rest upon discovery of the virus responsible in the throat washings from ill patients or upon detection of a rise of specific antibodies in the blood during convalescence. Antibody studies by means of complement fixation or neutralization tests have been found reliable in indicating infection with the known viruses (11, 13, 14). On the basis of etiology, a classification of influenza has been suggested (11, 12). Influenza A will indicate the disease caused by the virus isolated by Smith, Andrewes and Laidlaw in 1933, subsequently termed human influenza virus, epidemic influenza virus and now influenza virus A. Since 1933 many strains of this virus have been found in association with widespread outbreaks of influenza in many parts of the world which have occurred at intervals of about two years (6 to 11). In 1940, Francis (3, 6) and Magill (4, 5) isolated a virus which was antigenically dissimilar from virus A and proved it to be the cause of outbreaks of influenza in the United States in that year. Influenza B indicates the disease caused by this virus. Francis has shown that the epidemic of 1936 in California and elsewhere in the United States was

influenza B. The cases of clinical influenza for which no etiology can be established by appropriate tests are designated influenza X. Several undiscovered virus agents may be included in this group.

The knowledge that influenza comprises a group of clinically similar diseases caused by several serologically distinct viruses is of epidemiological significance, especially when the problem of prevention by vaccination is considered. It seems important to study carefully epidemics of influenza in the hope of developing means of early differentiation of types and of establishing the cause of each epidemic either by isolation of the virus or serological tests. We present observations made during an epidemic of influenza A which occurred in San Francisco in November and December 1940 and January 1941. The clinical features of the disease and the results of prophylactic inoculation against influenza A are described. The results were controlled by antibody studies on many individuals.

## METHOD

Blood specimens were taken during the acute stage and again two to three weeks later from 70 patients with clinical influenza who were admitted to the University of California Hospital. Complement fixation tests against influenza A were done on all specimens and neutralization tests on most of them. Neutralization tests with influenza B were made on the serums of all patients whose specimens failed to reveal a rise in antibodies to influenza A. The methods used have been described in detail elsewhere (13, 14, 18). One thousand minimal lethal doses were used in the neutralization tests. These tests were performed in the Research Laboratory of the California State Department of Public Health. The appearance of antibodies to influenza A was considered demonstrated if complement fixation or neutralization titers, or both, showed a definite increase between acute and convalescent specimens. Patients whose blood showed this increase are referred to as positive for influenza A and it is assumed that they had influenza A. The majority showed

<sup>1</sup> This study was aided by a grant from the Christine Breon Fund for Medical Research, University of California Medical School.

<sup>2</sup> Operated with the support and under the auspices of the International Health Division of the Rockefeller Foundation.

a four-fold or greater increase in titer by either or by both tests. Four patients had a two-fold rise by complement fixation test and one of these had a two-fold rise by neutralization test. These patients are included in the group positive for influenza A. A complete analysis of the antibody studies is reserved for a separate report (15).

The inoculation experiments were carried out with the complex vaccine of Horsfall and Lennette which was supplied through the courtesy of Dr. F. L. Horsfall, Jr. of the International Health Division of the Rockefeller Foundation (16, 17, 18). It was prepared from chick embryos inoculated with influenza A and canine distemper viruses (18). The desiccated material was rehydrated by adding 55 cc. of sterile distilled water to 25 cc. of dried vaccine immediately before inoculation. Inoculations were made by injecting 1 cc. of the resulting suspension subcutaneously. A single injection was employed in every instance.

#### EPIDEMIOLOGY

The group studied consists of the population of the University of California Medical Center in San Francisco. It includes medical, dental, pharmacy and nursing students, members of the nursing and house physician staffs, technicians and secretaries of the teaching departments, and members of the various maintenance services. It represents nearly all persons who spend full time at the Medical Center and consists of a total of 1213 individuals.

Influenza A appeared in the Hawaiian Islands on September 15, 1940, and reached epidemic proportions on about October 10, 1940.<sup>3</sup> In November, a little over a month after its appearance in Hawaii, cases of the same disease occurred in San Francisco. The first patient was admitted to the University of California Hospital on November 23. From this date to January 4, 1941, 271 individuals of the Medical Center population of 1213, or 22 per cent, had a febrile disease of one or more days' duration. This incidence was ascertained by canvass of the various departments. The interpretation of this figure is complicated by the difficulties usually encountered in a survey by canvass and by the interference of prophylactic measures. Incidence is considered in more detail in the section on vaccination.

Eighty of the 271 patients were admitted to the University of California Hospital. These included 29 nurses and nursing students, 17 medical students, 12 physician staff members, 14 dental students, 5 pharmacy students and 3 secretaries and hygienists. Of these, 70 were studied for antibodies to influenza A. Those negative to type A virus were studied for antibodies to influenza B. Fifty-three, or 75 per cent, were positive for influenza A. None was positive for influenza B. The first case of influenza A occurred on November 23, 1940, and the last on January 4, 1941. The peak incidence occurred in the week of November 29 to December 6. Table I shows the spread of the epidemic as indicated by dates of onset of those patients on whom antibody studies were done.

<sup>3</sup> This epidemic was studied by one of us (M. D. E.).

TABLE I

*Dates of onset by weeks of influenza in patients on whom antibody studies were done*

Weeks	Total number of patients		Inoculated patients	
	Positive for influenza A	Negative for influenza A and B	Positive for influenza A	Negative for influenza A and B
November 23 to 29, 1940.....	13	2	0	0
November 30 to December 6.....	23	2	7 <sup>14</sup>	1 <sup>1</sup>
December 7 to 13.....	7	5	20 <sup>11</sup>	30 <sup>10</sup>
December 14 to 20.....	8	3	1 <sup>2</sup>	21 <sup>2</sup>
December 21 to 27.....	1	3	0	20 <sup>2</sup>
December 28 to January 4, 1941..	1	2	1 <sup>1</sup>	1 <sup>1</sup>
Total.....	53	17	11	9
Per cent.....	75	25		

Superscripts indicate number of days after inoculation when onset of influenza occurred.

#### RESULTS OF INOCULATION WITH VACCINE

A total of 273 individuals of the Medical Center population was inoculated with the complex vaccine of Horsfall and Lennette. The injections were made on November 26 and 28 and December 3. The epidemic was in progress a few days before the first inoculations were carried out.

No significant reactions occurred following injection. Tenderness over the injected site, which persisted three to four days without redness or swelling, was a common experience. A few individuals had slight redness and swelling of the arm for twenty-four hours. One individual had generalized urticaria three days after injection and another had mild urticaria seven days afterwards. One experienced an erythematous eruption with swelling of hands, feet, and lips on the fifth day. Another suffered from rhinitis and sneezing for twelve hours beginning one hour after inoculation. None of these individuals had a history of allergic disease.

The incidence of influenza in vaccinated and control groups was compared (Table II). A febrile disease resembling influenza occurred in 23 of the 940 control subjects, an incidence of 2.5 per cent as ascertained by history. Thirty-six cases developed among 273 inoculated persons, an incidence of 13 per cent. The hospital staff group consisting of nurses, house physicians and medical laboratory personnel, was constantly under observation so that the information obtained about this smaller group is reliable. It consisted of 342

TABLE II

*Clinical influenza. Incidence and results of prophylactic inoculation*

Groups	Total	Not Inoculated			Inoculated		
		Number	Influenza	Per cent	Number	Influenza	Per cent
Service group*.....	416	383	113	29.5	33	6	18.1
Student group†.....	463	368	40	10.8	95	9	9.4
Hospital staff group‡...	334	189	82	43.4	145	21	14.5
Total population.....	1213	940	235	25.0	273	36	13.0

\* Includes members of service units, such as secretaries, technicians, librarians; and maintenance units, such as janitors.

† Includes medical, dental and pharmacy students.

‡ Includes nursing students and staff, house physicians and staffs of medical laboratories.

dividuals. Of 189 controls, 82, or 43 per cent, developed the disease. The incidence of influenza shown in Table II was much higher in the hospital control group than in the general control group, either because of better observation or because of greater exposure. The unvaccinated student group could be observed only if the individual members reported illness to the student infirmary. The Christmas holiday began during the decline in incidence of cases of influenza and the students left the institution during this period. Consequently, the incidence presented for the student group is inaccurate. The only figures in the survey subject to a significant error are those relative to the incidence among the general campus population, especially in the student group, who were not inoculated; all inoculated persons and the hospital staff group were observed. The general incidence of influenza at the Medical Center must have been higher than 25 per cent. Therefore, the difference in incidence between vaccinated and control groups is probably greater than that presented for the total population and may be more accurately represented by the experience of the hospital staff group.

Antibody studies were made on acute and convalescent blood specimens from patients of both groups. Fifty control and 20 vaccinated subjects who had influenza were studied. Forty-two (84 per cent) of the controls and 11 (55 per cent) of those inoculated showed significant rises in antibodies to influenza A and were considered positive

for influenza A (Table III). This further reduces the incidence of influenza A in the vaccinated group as compared to the controls.

Since influenza A appeared before vaccination was carried out, the days of onset must be compared in order to evaluate the effect of vaccination. Only established cases of influenza A will be considered. Table I shows the general incidence by weeks and indicates both control and vaccinated subjects. The number of days after inoculation when influenza A occurred in vaccinated persons are demonstrated in Table I by superscripts.

Of those inoculated who subsequently had influenza, only 4 were shown to have acquired influenza A more than ten days after inoculation. In 2 of these the disease developed twenty-three and thirty-nine days, respectively, and each devel-

TABLE III

*Results of antibody studies on control and inoculated cases of clinical influenza*

Group	Number of patients studied	Positive for influenza A	Per cent positive
Control.....	50	42	84
Inoculated.....	20	11	55
Total.....	70	53	75

oped significant increases in titer of both complement fixing and neutralizing antibodies during the disease. These 2 cases show clearly that vaccination did not afford certain protection. Table I shows that the incidence of influenza A in the epidemic reached its peak during the ten days after vaccination and then rapidly subsided in the population as a whole during the time when vaccination might be expected to become effective. It is unfortunate that the experiment was not begun sooner. However, the difference in incidence of influenza A in the vaccinated as compared to the control group is striking and may be due to a protective effect of vaccination which began sooner after inoculation than is usually considered likely.

#### CLINICAL FEATURES

The clinical characteristics described are based on the observation of patients in the hospital who were subsequently shown to have suffered from either influenza A, or neither influenza A nor B, as established by antibody studies. As indicated be-

fore, this group consisted of 70 persons, 53 of whom had influenza A and 17 neither influenza A nor B. Of these, 20 had been previously vaccinated and the characteristics of influenza A in them are also considered. Table IV summarizes the incidence of various symptoms. Table V in-

TABLE IV

*Frequency of symptoms in patients with influenza of known and unknown etiology*

Symptoms	Influenza A				Neither influenza A nor B	
	Con-trols	Vacci-nated	Total	Per cent	Vacci-nated and con-trols	Per cent
Chills or chilliness. . . . .	26	3	29	56.6	6	35.2
Headache. . . . .	29	8	37	69.8	14	82.3
Body pains. . . . .	37	9	46	86.7	16	94.1
Cough. . . . .	31	8	39	73.5	5	29.4
Nasal congestion. . . . .	17	4	21	39.6	5	29.4
Sore throat. . . . .	18	7	25	47.1	8	47.0
Hoarseness. . . . .	3	2	5	9.4	2	11.7
Lacrimation. . . . .	4	2	6	11.3	2	11.7
Photophobia. . . . .	3	1	4	7.5	0	0
Earache. . . . .	3	0	3	5.6	0	0
Dizziness. . . . .	1	0	1	1.8	0	0
Nausea. . . . .	5	1	6	11.3	5	29.4
Vomiting. . . . .	4	1	5	9.4	4	23.5
Abdominal pain. . . . .	3	0	3	5.6	2	11.7
Diarrhea. . . . .	0	2	2	3.7	2	11.7
Total cases. . . . .	42	11	53		17	

dicates the duration of fever, maximum temperature and maximum leukocyte counts, respectively, in the three groups. Influenza A in this epidemic was explosive in onset and severe during the height of the fever. Convalescence was rapid in most cases. A comparison of the symptoms experienced by patients with influenza A and by those with influenza of unknown etiology fails to reveal a significant difference (Table IV). Cough was more often present in patients with influenza A. In both groups general symptoms and symptoms of upper respiratory tract inflammation were predominant. In no case was the nasal congestion mentioned in the table suggestive of coryza. Sore throat was usually accompanied by a prominence of lymphoid follicles on the pharyngeal wall but not by a diffuse inflammation. Earache, when it occurred, was due to myringitis rather than to otitis media.

The duration of fever did not differ significantly in the two types of influenza (Table V). Ninety

per cent of the patients with influenza A had a febrile period of two to five days, and 68.5 per cent had a febrile period of three to four days. Those with influenza of unknown etiology suffered fever for from one to five days in 93.6 per cent and for three to four days in 50 per cent. The table indicates that patients with influenza A had significantly higher maximum temperatures

TABLE V

*Duration of fever, maximum temperature and maximum leukocyte count in patients with influenza of known and unknown etiology*

	Influenza A				Neither influenza A nor B	
	Con-trols	Vacci-nated	Total	Per cent	Vacci-nated and con-trols	Per cent
DURATION OF FEVER						
days						
1	0	0	0	0	1	6.2
2	1	0	1	1.9	3	18.7
3	11	3	14	27.4	4	25.0
4	17	4	21	41.1	4	25.0
5	8	2	10	19.6	3	18.7
6	1	0	1	1.9	1	6.2
7	2	0	2	3.9	0	0
9	0	1*	1*	1.9	0	0
12	1†	0	1†	1.9	0	0
Total cases	41	10	51		16	

MAXIMUM TEMPERATURE

degrees Centigrade						
37.0-37.5	—	1	1	1.9	4	26.6
37.5-38.0	3	—	3	5.8	6	40.0
38.0-38.5	12	—	12	23.5	1	6.6
38.5-39.0	20	7	27	52.9	2	13.3
39.0-39.5	5	2	7	13.7	1	6.6
39.5-40.0	1	—	1	1.9	1	6.6
Total cases	41	10	51		15	

MAXIMUM LEUKOCYTE COUNT

thousands per cubic millimeter						
0-5	9	2	11	22.6	4	33.3
5-7	15	5	20	40.8	4	33.3
7-10	10	1	11	22.6	1	8.3
10-15	3	2	5	10.2	1	8.3
15-20	1†	1*	2	4.0	2	16.6
Total cases	38	11	49		12	

\* Complicated by pneumococcus Type IV lobar pneumonia.

† Complicated by atypical pneumonia.

than those with influenza of unknown etiology. The maximum leukocyte counts as tabulated show little difference between the groups. Maximum counts were below 7,000 per cubic millimeter in 63.4 per cent and below 10,000 per cubic millimeter in 86 per cent of the cases of influenza A. Pulmonary complications occurred in 2 patients, both of whom were shown to have had influenza A. Pneumococcus Type IV lobar pneumonia developed in a 20-year-old student nurse after four days of fever with clinical and serological evidence of influenza A beginning December 19. Blood culture was negative. The patient recovered during sulfathiazole therapy. She had been inoculated with vaccine twenty-three days prior to onset of influenza A. At the onset of pneumonia, her leukocyte count increased from 5,600 to 20,000 per cubic millimeter. The second patient was a 27-year-old dental student who had evidence of atypical pneumonia in both lung bases on the fourth day of fever in the course of influenza A which had begun on December 6. He experienced a total febrile period of twelve days. The leukocyte count rose from 6,100 to 11,000 per cubic millimeter on the seventh day, to 16,470 per cubic millimeter on the tenth day. No causative organism was discovered in sputum or blood and no effect on the course was observed during the exhibition of sulfathiazole. It is probable that this patient had atypical pneumonia of virus etiology, possibly due to the virus of influenza A. He also made an uneventful recovery. He had not been inoculated with vaccine.

The characteristics of influenza A observed during this epidemic were similar to those in epidemics previously described (7, 8, 25). The disease was of moderate severity, the incidence of pulmonary complications was low, and no deaths occurred. We did not find it possible to distinguish clinically between influenza A and influenza of unknown etiology which occurred during the same epidemic. There was no evidence that the disease was modified in persons who had previously received a prophylactic inoculation. Subclinical infection may have influenced the results of antibody studies. This was not determined.

#### DISCUSSION

Since influenza of known etiology has been divided into two types, A and B, each caused by a

different virus agent, much of the confusion concerning the epidemiology of the group of diseases called influenza has been dispelled. That all cases of influenza are not caused by known agents seems clear. Influenza X may represent a variety of agents of virus or possibly other nature. In the group reported, a high proportion of cases were influenza A. Nevertheless, a definite proportion of cases appeared simultaneously on which tests failed to reveal evidence of infection with either virus A or B. An analysis of these cases failed to reveal an accurate clinical method of differentiation. All were clinical influenza, appearing and disappearing at the same time. Therefore, influenza A and a febrile respiratory disease of unknown etiology occurred together in this epidemic. Influenza B was not discovered.

Since the discovery of the virus etiology of one form of influenza (1), studies on the effectiveness of vaccination with active and inactive virus have been made in animals and humans (19 to 30). The parenteral inoculations of both active and inactive material by either single or multiple injections have been made by the intraperitoneal, intramuscular, subcutaneous and intradermal routes. In ferrets, mice and humans, rises in antibodies have been induced by vaccination. In ferrets and mice relative immunity has developed to subsequent infection which is to a great extent strain-specific and which persists three to four months (16, 17, 19, 22, 27). Several experiments have been carried out in which human volunteers were inoculated subcutaneously or intramuscularly with active or inactive virus suspensions and subsequently observed during an epidemic of influenza A (23 to 25, 28 to 30). None of these experiments has resulted in conclusive proof of the effectiveness of vaccination of human beings against influenza A. In all of them evaluation has been made difficult by various factors, such as a low attack rate in controls, too early appearance of the epidemic after vaccination, small number of subjects involved, or paucity of tests on throat washings or blood specimens. In two studies (24, 25), virus was obtained from patients who had been previously vaccinated. Recent inoculation experiments with the complex vaccine of Horsfall and Lennette have given some evidence that a protective effect against influenza A was obtained

(30). In another study in California during the recent epidemic of influenza A, the formalin-inactivated complex vaccine and a living vaccine of influenza A virus appeared to give comparable results. The results were suggestive of a protective effect against influenza A after inoculation with either preparation at one institution but were questionable at another (29).

The observations we present are also difficult to evaluate and do not give conclusive evidence of protection against influenza A by vaccination. This was due to the presence of cases of influenza A in the community before inoculations were begun and to the fact that the peak incidence was reached within ten days after inoculation of most individuals. Furthermore, two vaccinated individuals acquired influenza A twenty-three and thirty-nine days, respectively, after inoculation. However, the incidence of influenza in vaccinated individuals was markedly less than in the controls. In addition, the disease which occurred in vaccinated persons was proved to be influenza A by antibody studies in a much smaller percentage of those inoculated than among the control group. The definite difference in incidence of disease between inoculated persons and controls is difficult to explain unless we assume that some protection was afforded by the vaccine. If this occurred, the vaccine must have produced an immunity earlier after inoculation than is usually considered possible and at a time when measurable protective antibodies are absent or just beginning to rise in the blood. The factors of age, nutrition and previous exposure to influenza A may be of significance in determining the speed with which antibody formation occurs and protection develops. These factors may also explain differences in results of vaccination of different groups with the same vaccine. The other alternative in the present study is to consider that the inoculated group escaped infection by chance, which is, however, statistically unlikely.

#### SUMMARY

1. Clinical and epidemiological observations made during an epidemic of influenza A are presented.

2. Antibody studies indicated that influenza A was the causative agent in 75 per cent of the cases.

There was no evidence of the presence of influenza B.

3. Subcutaneous inoculation of 273 of a total of 1213 individuals was performed with a mixed influenza A—distemper vaccine. A marked reduction in incidence of influenza A occurred in the vaccinated group as compared to the controls. The incidence of influenza in the control groups was between 25 and 43 per cent as compared to 13 to 15 per cent in those inoculated. These observations suggest that a measure of protection was afforded by vaccination but do not permit the conclusion that efficient protection resulted.

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# CIRCULATORY FAILURE IN ACUTE INFECTIONS

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Failure of the circulation in acute infectious diseases produces a clinical picture that resembles in many ways that seen in hemorrhage, traumatic shock, or nitrite collapse. The patients show narrowing of the field of consciousness, pallor, sweating, cold extremities, rapid feeble pulse, collapsed veins, and low arterial pressure. The failure of the circulation in hemorrhage and traumatic shock has been shown to be caused by a diminished blood volume. Transfusion of blood or plasma, therefore, restores the circulation. In collapse which occurs in subjects in the upright position after the ingestion of sodium nitrite, circulatory failure is caused by pooling of blood in dilated veins and venules in the lower part of the body. Hence, returning the subject to the horizontal position restores the circulation. The purpose of this paper is to describe the changes in the circulation which occur in the so-called "medical shock" produced by certain acute infections, and to determine whether a diminished blood volume or venous pooling is the primary factor in the production of this type of circulatory failure.

## METHOD

Before concluding that a diminished blood volume is of primary importance in producing a given type of circulatory failure, it is necessary to demonstrate (a) that the blood volume is decreased, and (b) that the circulatory failure will not occur if the decrease in blood volume is prevented. In previous experiments on an unselected group of dying patients with terminal circulatory failure, a decrease in plasma volume was frequently found (1). From this data alone it was not possible to determine whether the change in plasma volume was really a significant factor in the circulatory failure, or whether it was merely an incidental finding secondary to the disease process itself, or to an inadequate fluid intake or to malnutrition. In the present study, therefore, more emphasis has been placed on the response of the circulation to transfusion than on the measurement of the plasma volume. In studying the response of the circulation to transfusion, it was essential to demonstrate that the volume of blood had been increased effectively, and that fluid had not left the blood stream as fast as it was given. This was done

in two ways: (1) by following the hematocrit reading, the serum protein concentration, and the plasma volume, and (2) by administering fluid until the pressure was increased in the large veins. The plasma volume was determined by the method of Gibson and Evans (2), as adapted to the photoelectric colorimeter (3). A 1.6 per cent solution of potassium oxalate was used for the hematocrit determinations. The serum protein determinations were performed by the falling drop method of Kagan (4). The hemoglobin concentration was measured with the photoelectric colorimeter (5) or by the method of Sahli. Venous pressure measurements were made by the method of Moritz and von Tabora (6). The femoral or external jugular veins were used in most cases, as it is difficult to obtain an accurate venous pressure measurement from the arm veins when there is marked peripheral vasoconstriction. Arterial pressures were determined by the use of a mercury manometer and auscultation or palpation.

## OBSERVATIONS

Eight cases of circulatory failure in acute infectious diseases were studied (Table I). There were 6 cases of pneumococcus pneumonia, one case of hemolytic streptococcus septicemia complicating a urethral stricture and chronic pyelonephritis, and one case of staphylococcus septicemia. Six of the 8 cases had a bacteremia. All the patients studied presented a similar clinical picture. They were stuporous or comatose. The rectal temperatures ranged from 97° to 106.4° F. The skin was pale and often covered with perspiration. The extremities were cold, and this finding usually preceded the fall in arterial pressure. The skin of the body was usually warm, although in the terminal stages it too became cool. The radial pulse was feeble or impalpable. The pulsations in the femoral artery were more prominent than those in the radial artery. In the patients with pneumonia the respirations were usually rapid and deep, and tracheal râles were often present. The respirations were normal in Case 7 who had hemolytic streptococcus septicemia.

In all the cases included in the study, there was a fall in arterial pressure. The systolic and dias-

TABLE I  
*Observations on eight patients with circulatory failure produced by acute infections*

Observations on eight patients with circulatory failure produced by acute infections															
Case	No.	Diagnosis	Date, time	General observations	Procedure	Rectal temperature	Heart rate	Arterial pressure	Veinous pressure	Plasma volume	Normal plasma volume for height	Serum protein concentration	Hematocrit reading	Hemoglobin concentration	Electrocardiogram
1	♂	Lobar pneumonia. Sputum type III pneumococcus. Blood culture type III pneumococcus.	March 18, 1940 3 p.m. 9 p.m. 10 p.m. 10:05 10:10 11 p.m. 11:30 11:33 March 19, 1940 12:07 a.m. 12:10 April 17, 1940 3 p.m. 10 p.m. April 18, 1940 10 a.m. 12:25 p.m. 4:15 p.m. 5:10 p.m. 5:18 5:20 5:30 7:30 p.m. January 15, 1941 5 p.m. January 16, 1941 10 a.m. 11:40 a.m. 11:50 12:25 p.m. 1 p.m. 1:30	Third day of disease. History of chronic alcoholism. Patient was conscious. Skin was hot and dry. Radial pulse was normal. Stuporous. Hands and legs cold. Patient perspiring freely. Radial pulse weak. Veins of neck not distended.  Stuporous. Hands and feet cold.  Veins of arm more prominent. No change in clinical condition.  Dead. Autopsy obtained.  Patient was weak but conscious. Skin was warm, moist and flushed.  Patient comatose. Extremities cold and cyanotic, and covered with perspiration. Radial pulse not palpable. Neck veins not distended.  Neck veins distended. Radial pulse not palpable.  No change in appearance except for increased prominence of veins.  Dead. Autopsy obtained.  Sixth day of disease. Patient conscious, cyanotic. Skin cold and covered with perspiration.  Skin cold and cyanotic. Patient very weak.  Dead. Autopsy obtained.	Paredrinol sulphate, 20 mgm. intravenously. After paredrinol. Transfusion of 1100 cc. of blood. Completed at 11:30 p.m.  Paredrinol sulphate, 20 mgm. intravenously.  20 mgm. paredrinol intravenously.  Transfusion of 600 cc. of blood.  Transfusion of 500 cc. of blood.  Paredrinol sulphate, 40 mgm. intravenously.  Intravenous 10% glucose and saline started. Paredrinol sulphate no longer effective in causing a rise in arterial pressure.  500 cc. of plasma intravenously followed by 500 cc. of 10% glucose in saline. Infusion completed.	F. 105.4 104.0 102.5 134 102.6 114 110 103 99 97 140 132 102 130 106.4 106.4 156 154 144	150 120 120 134 114 110 120 120 120 not obtainable not obtainable ? 80/65 ? 105/65 75 by palpation 60/50 80/50 76/50	175/105 84/70 68/52 140/84 70/50 70/56 120/70 60/45 110/75 not obtainable not obtainable ? 16.5 16.5 3 5 6.5	cm. of water 2610 3100 3680 3500 3500 2760 2800 6.6 6.3 41.4	grams per cent 46.0 48.5 6.3 6.7 6.8 13.5 51 6.3 41.4	grams per cent 12.8 8 44 Normal.	Normal.			
70	♂	Lobar pneumonia. Sputum type 9 pneumococcus. Blood culture type 9 pneumococcus. Chronic rheumatoid arthritis.	March 19, 1940 12:07 a.m. 12:10 April 17, 1940 3 p.m. 10 p.m. April 18, 1940 10 a.m. 12:25 p.m. 4:15 p.m. 5:10 p.m. 5:18 5:20 5:30 7:30 p.m. January 15, 1941 5 p.m. January 16, 1941 10 a.m. 11:40 a.m. 11:50 12:25 p.m. 1 p.m. 1:30	Third day of disease. History of chronic alcoholism. Patient was conscious. Skin was hot and dry. Radial pulse was normal. Stuporous. Hands and legs cold. Patient perspiring freely. Radial pulse weak. Veins of neck not distended.  Stuporous. Hands and feet cold.  Veins of arm more prominent. No change in clinical condition.  Dead. Autopsy obtained.  Patient was weak but conscious. Skin was warm, moist and flushed.  Patient comatose. Extremities cold and cyanotic, and covered with perspiration. Radial pulse not palpable. Neck veins not distended.  Neck veins distended. Radial pulse not palpable.  No change in appearance except for increased prominence of veins.  Dead. Autopsy obtained.  Sixth day of disease. Patient conscious, cyanotic. Skin cold and covered with perspiration.  Skin cold and cyanotic. Patient very weak.  Dead. Autopsy obtained.	Paredrinol sulphate, 20 mgm. intravenously. After paredrinol. Transfusion of 1100 cc. of blood. Completed at 11:30 p.m.  Paredrinol sulphate, 20 mgm. intravenously.  20 mgm. paredrinol intravenously.  Transfusion of 600 cc. of blood.  Transfusion of 500 cc. of blood.  Paredrinol sulphate, 40 mgm. intravenously.  Intravenous 10% glucose and saline started. Paredrinol sulphate no longer effective in causing a rise in arterial pressure.  500 cc. of plasma intravenously followed by 500 cc. of 10% glucose in saline. Infusion completed.	F. 105.4 104.0 102.5 134 102.6 114 110 103 99 97 140 132 102 130 106.4 106.4 156 154 144	150 120 120 134 114 110 120 120 120 not obtainable not obtainable ? 80/65 ? 105/65 75 by palpation 60/50 80/50 76/50	175/105 84/70 68/52 140/84 70/50 70/56 120/70 60/45 110/75 not obtainable not obtainable ? 16.5 16.5 3 5 6.5	cm. of water 2610 3100 3680 3500 3500 2760 2800 6.6 6.3 41.4	grams per cent 46.0 48.5 6.3 6.7 6.8 13.5 51 6.3 41.4	grams per cent 12.8 8 44 Normal.	Normal.			
48		Lobar pneumonia. Sputum type III pneumococcus. Blood culture negative.	January 15, 1941 5 p.m. January 16, 1941 10 a.m. 11:40 a.m. 11:50 12:25 p.m. 1 p.m. 1:30	Third day of disease. History of chronic alcoholism. Patient was conscious. Skin was hot and dry. Radial pulse was normal. Stuporous. Hands and legs cold. Patient perspiring freely. Radial pulse weak. Veins of neck not distended.  Stuporous. Hands and feet cold.  Veins of arm more prominent. No change in clinical condition.  Dead. Autopsy obtained.  Patient was weak but conscious. Skin was warm, moist and flushed.  Patient comatose. Extremities cold and cyanotic, and covered with perspiration. Radial pulse not palpable. Neck veins not distended.  Neck veins distended. Radial pulse not palpable.  No change in appearance except for increased prominence of veins.  Dead. Autopsy obtained.  Sixth day of disease. Patient conscious, cyanotic. Skin cold and covered with perspiration.  Skin cold and cyanotic. Patient very weak.  Dead. Autopsy obtained.	Paredrinol sulphate, 20 mgm. intravenously. After paredrinol. Transfusion of 1100 cc. of blood. Completed at 11:30 p.m.  Paredrinol sulphate, 20 mgm. intravenously.  20 mgm. paredrinol intravenously.  Transfusion of 600 cc. of blood.  Transfusion of 500 cc. of blood.  Paredrinol sulphate, 40 mgm. intravenously.  Intravenous 10% glucose and saline started. Paredrinol sulphate no longer effective in causing a rise in arterial pressure.  500 cc. of plasma intravenously followed by 500 cc. of 10% glucose in saline. Infusion completed.	F. 105.4 104.0 102.5 134 102.6 114 110 103 99 97 140 132 102 130 106.4 106.4 156 154 144	150 120 120 134 114 110 120 120 120 not obtainable not obtainable ? 80/65 ? 105/65 75 by palpation 60/50 80/50 76/50	175/105 84/70 68/52 140/84 70/50 70/56 120/70 60/45 110/75 not obtainable not obtainable ? 16.5 16.5 3 5 6.5	cm. of water 2610 3100 3680 3500 3500 2760 2800 6.6 6.3 41.4	grams per cent 46.0 48.5 6.3 6.7 6.8 13.5 51 6.3 41.4	grams per cent 12.8 8 44 Normal.	Normal.			

TABLE 1—Continued

Case	Age	Diagnosis	Date, time	General observations	Procedure	Rectal temperature	Heart rate	Arterial pressure	Veinous pressure	Plasma volume	Normal plasma volume for height	Serum protein concentration	Hemoglobin concentration	Hematocrit	Electrocardiogram
	Years					F.		mm. of Hg.	cm. of water	cc.		grams per cent	grams per cent		
4	80	Chronic bronchitis, Bronchopneumonia. Sputum types 3 and 20 pneumococci.	January 22, 1911	Fourth day of illness. Patient conscious; appears acutely ill.		100.5	130	130/80		2	3000	7.1	52.1		Normal.
			January 23, 1911 10:10 a.m.	Patient comatose, cyanotic. Extremities cold and moist. Profuse perspiration over body. Veins not distended.	500 cc. plasma intravenously. 400 cc. 10% glucose and saline intravenously. 250 cc. plasma		130	62/50	0.5			0.8	47.1		
			10:30	Hands warmer. Radial pulse weak.				70/50							
			11:15 a.m.					80/50							
			12:00 M.					76/10							
			12:15 p.m.	Hands cold, perspiring. Radial pulse slightly stronger. Neck veins prominent and distended. Face cyanotic and plethoric.		102	90	78/10		11		0.6	42.8		
			12:30												
			12:35	Died.											
5	65	Lobar pneumonia. Sputum type III pneumococcus. Blood culture type III pneumococcus. Diabetes mellitus.	December 29, 1910	Patient acutely ill. Pulse normal. Veins not distended.	Intravenous injection of 2000 cc. of 45% glucose in saline just completed.	103	110	115/85					12.1		Normal.
			December 30, 1910 2 p.m.	Patient rational. Hands cool and sweating. Pulse of good volume.	1000 cc. of 10% dextrose in water begun.	102.8									
			5:30 p.m.				122	135/70							
			8 p.m.	Patient pale. Hands too cold. Patient somewhat stuporous. Not sweating.											
			7 p.m.	Attack of pulmonary edema treated by tourniquets and morphia.											
			10 p.m.	Patient pale. Hands and feet cool. No sweating. Neck veins not visible. Veins of hands still slowly when obstructed.		101.4	121	100/60	9	2760	2250	7.4	41.9		
				Improved. More alert but still weak. Hands warmer. Heart grossly irregular.			145	130/70	11	2080		7.5	40		Auricular fibrillation.
			December 31, 1910	Peripheral circulation appears normal.		100.5									
			January 1, 1911	Recovered from pneumonia, but later died, January 6, 1911, as a result of cerebral thrombosis. Autopsy obtained.		101		125/70							
6	50	Lobar pneumonia. Sputum type III pneumococcus. Blood culture type III pneumococcus. Pulmonary tuberculosis.	March 5, 1911	Fifth day of disease. Patient undernourished and acutely ill.		101.6	128	122/78					48		
			March 6, 1911 2 p.m.	Patient but weak. Hands and forearms cool. Radial pulse feeble.		101	130	80/66							
			5 p.m.	Patient stuporous, but could be aroused. Pale. Hands and forearms cool, pulse feeble, veins not distended.		103.8	144	90/68	0	3320	3250	5.8	42.0		Normal.
			8 a.m.	Patient improved. Extremities warm. Skin slightly flushed.	Transfusion of 500 cc. of blood.	100.0	100	100/80	7				43		
			March 8, 1911	Mentally alert. Hands warm. Patient recovered without showing any further signs of circulatory insufficiency.		100.0	90	90/66	5	3370		0.3	43.3		



tolic pressures both decreased and the pulse pressure became narrower. The fall in arterial pressure was often marked in degree, and in some cases the arterial pressure could not be obtained either by auscultation or palpation in the later stages of the circulatory failure. The pulse rate was rapid. Two cases developed auricular fibrillation during the period of circulatory failure. The venous pressure was determined before the administration of blood or plasma in 5 cases. The values ranged from 0 to 8 cm. of water. Thus the venous pressure was not elevated in any of these cases. Electrocardiograms were normal in 5 cases, and showed auricular fibrillation in 2 cases.

Marked hemoconcentration was not present in any of the cases studied. The hematocrit readings before the administration of blood or plasma ranged from 26.5 to 52.1, and the serum protein concentrations ranged from 5.8 to 7.4 grams per 100 cc. Plasma volume determinations were made in all cases and showed no significant variation from the normal. In the 6 cases in which no significant anemia was present, the plasma volume determined before the administration of blood or plasma averaged 0.3 per cent above the normal value for the patient's height, as given by Gibson and Evans (2). The values for the plasma volume ranged from -19 to +22 per cent of the normal value for the height. In Case 2 the plasma volume determined before the onset of the circulatory failure, when the blood pressure was 110/75, did not differ significantly from the plasma volume determined when the patient had severe circulatory failure and the blood pressure could not be obtained. In Case 5, in whom temporary recovery occurred, and in Case 6 in whom permanent recovery occurred, there was no significant change in plasma volume after improvement of the circulation.

The effect of change in posture on the arterial pressure was determined in 4 cases. The arterial pressure was determined in the horizontal position, in the Trendelenburg position with the foot of the bed raised 18 inches off the floor, and with the patient sitting at an angle of 50 degrees. In Cases 3, 6 and 7, there was no difference in the arterial pressure in the different positions. In Case 4, the arterial pressure was 62/50 in the sitting position and 70/50 in the Trendelenburg position.

The effect of transfusions of blood or plasma on the arterial pressure and clinical condition was determined in 6 cases. Four patients (Cases 1, 2, 7, and 8) received 1000 to 1100 cc. of blood or plasma. One patient (Case 4) received 750 cc. of plasma, and another patient (Case 3) received 500 cc. of plasma. In addition, several patients were given a solution of 10 per cent glucose in saline intravenously (Table I). In none of the patients was there any definite improvement immediately after the administration of the blood or plasma. Case 7 improved temporarily, but this improvement did not begin until several hours after the transfusion. The effect of transfusions on arterial pressure was slight. In 2 patients there was no change in arterial pressure after transfusion. In 3 patients there was a rise in systolic arterial pressure of 5 to 10 mm. of mercury. In one patient the arterial pressure continued to fall during the transfusion. In 4 patients the venous pressures before and after transfusion were compared. An increase in venous pressure occurred in all subjects. The increase ranged from  $3\frac{1}{2}$  to 8 cm. of water and averaged 7 cm. of water. In one patient (Case 2), the venous pressure was measured only after transfusion. In this patient the venous pressure appeared clinically to be normal before transfusion. After transfusion, clinically it appeared elevated and measured  $16\frac{1}{2}$  cm. of water.

In the 3 patients receiving plasma (Cases 3, 4, and 8), there was a lowering of the hematocrit reading after the administration of the plasma, indicating that an increase in plasma volume had occurred. In 2 patients who received whole blood (Cases 2 and 7), plasma volume and hematocrit readings were determined after transfusion. In these cases there was a slight decrease in plasma volume and a moderate increase in hematocrit reading. In persons with an adequate circulation, similar changes occurred after transfusion.

Two patients in the study (Cases 1 and 2) were given paredrinol sulphate intravenously. In both cases there was a striking rise in arterial pressure which lasted approximately 20 minutes. In these patients the radial pulse became stronger, but their general conditions appeared the same. In both cases injections of paredrinol sulphate, given shortly before death, had no effect. These

observations show that at a time when the circulation was not benefited by transfusion, it was still able to respond to an adequate stimulus.

The ulnar nerve at the elbow was injected with novocain in Cases 2 and 8. At the time of injection, the extremities were cold, the radial pulse was thready or impalpable, and the blood pressure had begun to fall. In each subject the ulnar side of the hand, the little and ring fingers became warmer than the other fingers of the same hand or than the fingers of the opposite hand. The difference in temperature was unmistakable and was confirmed by several observers who did not know that the ulnar nerve had been injected.

#### DISCUSSION

All the patients selected for study had circulatory failure characterized not only by a marked fall in arterial pressure, but also by a decrease in peripheral blood flow as shown by pallor, cold extremities and collapsed veins. A fall in arterial pressure alone cannot be used as a criterion for selecting cases of circulatory failure, because in many instances a moderate decrease in arterial pressure may occur without any signs of circulatory insufficiency; in rarer cases, even a striking fall in arterial pressure may not be accompanied by a great decrease in peripheral blood flow. It must also be remembered that in many patients dying of acute infection, the circulation is adequate but death results from other causes, such as respiratory failure or aspiration of vomitus.

Failure of the circulation of the type described here is most commonly seen in overwhelming infection associated with bacteremia. It occurs most frequently in the older age groups or in persons who are poorly nourished because of chronic disease or inadequate intake of food.

Patients with acute infection and circulatory failure of the type described here clinically resemble in many ways cases of traumatic shock or hemorrhage. In both there are signs and symptoms of diminished peripheral blood flow and tissue anoxia. For this reason it has been suggested that circulatory failure in acute infections, in traumatic shock, and in hemorrhage, has the same etiology—namely, a diminished blood volume (7, 8, and 9). Eppinger and Schurmeyer (7) stated that the circulating blood volume, as measured by

the carbon monoxide method, is decreased in shock associated with acute infectious diseases. They attributed the circulatory failure to a decreased venous return to the heart. Andrews and Harkins (9) weighed the lungs in patients dying of pneumonia, and suggested that the circulatory failure resulted from the loss of plasma into the lungs with a consequent decrease in plasma volume. The data reported here indicate that the circulatory failure seen in acute infections differs in two essential ways from that of hemorrhage or traumatic shock; (a) laboratory studies do not show any evidence of a diminished blood volume or of hemoconcentration, and (b) transfusions are not an effective form of therapy. It must be remembered that in the cases studied here, the fluid intake was maintained either by mouth or by the parenteral administration of fluid. Undoubtedly, in patients with diarrhea or vomiting who have not received sufficient parenteral fluid, dehydration and hemoconcentration may play an important part in producing circulatory insufficiency, and the administration of fluid will cause improvement. This paper is concerned with patients in whom circulatory failure has occurred in spite of an adequate fluid intake.

It has been suggested that failure of the vasomotor center is responsible for the circulatory failure in acute infectious diseases (10). Investigation in experimental animals has shown that the vasomotor center continues to function in circulatory failure caused by hemorrhage and traumatic shock (11). Porter (12) concluded from his investigations in animals that the vasomotor center functions normally in acute infections. In the cases reported here, the development of cold extremities in the presence of a high rectal temperature and before a marked fall in arterial pressure had occurred indicates that there was a diminution in peripheral blood flow. That this decrease in blood flow was due in part to neurogenic vasoconstriction was demonstrated in Cases 2 and 8 by the fact that the ulnar side of the hand and the little finger became warmer after neurogenic impulses were removed by block of the ulnar nerve at the elbow. While these experiments show that vasoconstrictor impulses were reaching the vessels of the hand, they do not prove that the functions of the vasomotor center were necessarily normal.

It has been thought that the circulatory failure in acute infections may be the result of pooling of blood in dilated capillaries and veins, so that the venous return to the heart becomes inadequate (13, 14). This type of circulatory failure can be produced in the laboratory by motionless standing. Because of gravity, the blood accumulates in the dependent portions of the body, the venous return to the heart becomes inadequate, and the circulation fails. When the subject is placed in the horizontal or Trendelenburg position, blood flows back from the dilated capillaries and venules, the venous return to the heart becomes adequate, and the circulation rapidly returns to normal. In the cases studied here, the failure of the circulation to improve in the Trendelenburg position indicates that the circulatory failure was not the result of pooling of blood in capillaries and venules which could be drained towards the heart by elevation of the lower portion of the body. It might still be argued that the blood was trapped in the smaller vessels and that even with the aid of gravity the blood could not reach the great veins, where gravity would be effective in increasing the venous return to the heart. That this was not the case was shown by transfusion of blood and plasma and by infusion of 10 per cent glucose in saline. Adding fluid to the vascular bed of sufficient amount to cause distension of the superficial veins and an average rise in pressure of 7 cm. of water in the femoral or external jugular veins did not cause the circulation to improve significantly. In these cases there was no question of trapping blood in the periphery.

Although the data reported here indicate that peripheral pooling of blood is not the primary mechanism in producing circulatory failure, there is considerable evidence that the tone of the small blood vessels is altered. Acute respiratory infections are known to produce postural fainting, presumably through a loss of venous tone (15). Similar observations have been made in patients with pneumonia (16). Other investigators (17, 18) have studied the tone of the small vessels of the skin in pneumonia by measuring the height to which the venous pressure must be raised to obliterate an area of blanching produced by pricking epinephrine into the skin. They concluded that the tone of the small vessels of the skin is decreased.

In recent years, little emphasis has been placed on the heart as a factor in circulatory failure in acute infectious diseases because of the absence of the classical picture of congestive failure and because of the ineffectiveness of digitalis therapy (19). Certain experiments on animals also served to draw attention away from the heart. Romberg, Passler and others (10) demonstrated in rabbits with acute infections that the arterial pressure could be raised by pressure on the abdomen or by the administration of saline solution intravenously. From this they concluded that the heart was functioning normally. Newburgh and Porter (20) found that the hearts of dogs with pneumonia would contract normally when removed from the body. The fact that in the patients reported here the addition of fluid to the vascular system caused an average rise in venous pressure of 7 cm. of water without causing significant improvement in the circulation indicates that the heart was not functioning normally. None of the patients had an elevation of the systemic venous pressure before transfusion. In cases of pneumonia it is difficult to evaluate pulmonary congestion but, in the patient with streptococcus septicemia (Case 7), the roentgenogram of the lung showed no evidence of congestion. The fact that auricular fibrillation developed in 2 cases also suggests that the infection had a deleterious effect on the myocardium. The absence of venous congestion, however, demonstrates that other factors must also play an important rôle in this type of circulatory failure.

The data suggest that circulatory failure in acute infections is not produced by the failure of a single portion of the cardiovascular system, but the entire cardiovascular system appears to be damaged by the infection. It is unlikely that the lack of response to transfusions is due to permanent irreversible damage to the circulatory system secondary to the fall in arterial pressure. In Case 7, there was no significant response to transfusions although, when the infection subsided, a period of temporary improvement occurred. In the other patients studied, the observations were made shortly after the fall in arterial pressure began. From the experimental data it is impossible to state the exact sequence of events in circulatory failure in acute infections. A working hypothesis which is in accord with observed facts is as fol-



lows: The cardiac output falls because of damage to cardiac function, and compensatory vasoconstriction occurs in the arterioles. The tone of the veins and venules is decreased, and therefore the venous pressure is not elevated.

The only effective form of therapy in these cases has been that directed toward controlling the infection. In Case 7 in whom the infection subsided spontaneously, a period of temporary improvement occurred. In Case 6, in whom the infection was combated with sulfathiazole and large doses of antipneumococcal serum, the signs of circulatory failure disappeared. The same was true of Case 5, although this patient later died of a cerebral thrombosis which probably developed during the period of circulatory failure. Efforts directed towards the treatment of circulatory failure itself resulted in no significant improvement.

#### SUMMARY AND CONCLUSIONS

1. Eight patients with circulatory failure produced by acute infection were studied. There were 5 cases of lobar pneumonia, 4 of which had bacteremia. There was 1 case of streptococcal septicemia, 1 of staphylococcal septicemia, and 1 of bronchopneumonia without bacteremia. The circulatory failure was characterized by a decrease in peripheral blood flow and a fall in arterial pressure.

2. Measurements of the hematocrit level, the serum protein concentration, and the plasma volume, showed no evidence of significant hemoconcentration or of a diminished blood volume.

3. The venous pressure determined before transfusion was normal.

4. Elevating the foot of the bed did not improve the circulation.

5. Transfusions of whole blood, or plasma, or the infusion of 10 per cent glucose in saline until the venous pressure rose, did not produce any improvement in the circulation.

6. Blocking the ulnar nerve caused the ulnar side of the hand and the 4th and 5th fingers to become warmer than the other fingers. This showed that the vasoconstriction in the hand was neurogenic in origin.

7. The circulatory failure in these cases does not have the same mechanism as that of hemorrhage or traumatic shock, because the plasma volume is not decreased and transfusions are not beneficial.

It is not caused by venous pooling, because filling the venous system does not improve the circulation.

8. The entire cardiovascular system appears to be damaged by the infection. The absence of congestion, and the fact that the venous pressure is not increased, may be explained by simultaneous injury to the heart and loss of venous tone.

9. Improvement in the circulation occurs only when the infection is brought under control. Therapy should therefore be directed towards overcoming the infection rather than attempting to treat the circulatory failure itself.

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# STUDIES ON INTRAPULMONARY MIXTURE OF GASES. IV. THE SIGNIFICANCE OF THE PULMONARY EMPTYING RATE AND A SIMPLIFIED OPEN CIRCUIT MEASUREMENT OF RESIDUAL AIR

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In preceding papers of this series, we have studied (1) the relative effectiveness of mixing or distribution of tidal air into alveolar spaces in normal and abnormal subjects, and have proposed (2) an open circuit method for residual air determination, in which nitrogen in the lungs is washed out by continuous inhalation of pure oxygen.

The present paper (a) gives the results of a number of determinations of residual air, by the open circuit method, in normal subjects and patients with chronic cardiac or chronic pulmonary disease; (b) offers certain simplifications of technique in the open circuit method; and (c) suggests further significance of some of the data which this method provides.

It will simplify the succeeding discussion if a brief review is given of the theoretical basis of the open circuit method of residual air deter-

mination. The principle underlying all residual air methods which employ an inert gas, such as nitrogen or hydrogen, is the measurement of this inert gas before and after its distribution (by means of a period of breathing) between an unknown volume (the lungs) and a known volume (the spirometer). The relationship is described by a simple formula. Let nitrogen be the inert gas measured:

(Nitrogen percentage in lungs at start  $\times$  lung volume) + (nitrogen percentage in spirometer at start  $\times$  spirometer volume) = (nitrogen percentage in lungs at end  $\times$  lung volume) + (nitrogen percentage in spirometer at end  $\times$  spirometer volume).

For purposes of simplification, the correction factor due to nitrogen excreted from the body (3) is here disregarded.

Solving the above equation for lung volume,

$$\text{Lung volume} = \frac{(\text{N}_2 \text{ per cent in spirometer at end} \times \text{spirometer volume}) - (\text{N}_2 \text{ per cent in spirometer at start} \times \text{spirometer volume})}{(\text{N}_2 \text{ per cent in lungs at start}) - (\text{N}_2 \text{ per cent in lungs at end})}$$

The factors in the numerator can be determined accurately. The major error in measurement of residual air, or functional residual air (lung volume) is due to inaccuracy in estimating nitrogen concentration in the lungs in the presence of poorly aerated lung spaces—in other words, due to inaccurate alveolar sampling (or in some methods inaccurate assumptions regarding alveolar values).

On the other hand, any errors in the final value for lung volume, due to errors in estimating alveolar nitrogen concentration, will be minimized

if the denominator in the above formula can be made as large as possible. Since the alveolar nitrogen under conditions of breathing normal air is approximately the same ( $81 \pm 1$  per cent), it follows that a method which brings the alveolar nitrogen, at the end of the breathing period, to a value as near zero as possible, will, other things being equal, ensure the smallest error due to stagnant air in the lungs untapped by alveolar sampling. It is this which the open circuit method provides by emptying the lungs of their nitrogen as completely as possible through continuous inhalation of pure oxygen.

In addition to giving more accurate values for

<sup>1</sup> Working under a grant from the Commonwealth Fund.

residual air in patients with inadequate pulmonary aeration, the method permits certain estimates or approximations in alveolar values, with a minimum of error. This again is due to the large percentage difference between alveolar nitrogen at the start and that at the end of the breathing period. These approximations, providing a simplification of technique, will be discussed in a subsequent section.

Results of 158 duplicate determinations of residual air by the open circuit method

On 134 subjects, 158 duplicate residual air determinations of functional residual air were made under basal conditions. Each pair of measurements was made on the same morning, with a 15- to 30-minute interval between the two tests. The results of these paired determinations show that:

In 74 duplicate tests, or 46.8 per cent of the whole series, each determination deviated by less than 2 per cent from the mean of the pair of values; in 109 duplicate tests, this deviation was less than 3 per cent from the mean; in 139 duplicates, the deviation was less than 5 per cent; and in 156 of the 158 cases, the deviation was less than 7 per cent from the mean value of the pair.

It is of interest that there is a more or less even scatter of these deviations regardless of the size of the functional residual air.

While differences larger than 5 per cent are not satisfactory from the technical standpoint, it may be noted that the cases studied include a large proportion of advanced fibrosis and emphysema. The latter, by reason of the fundamental pathology present, offer great technical difficulties for any residual air method. It is also not unlikely that some of these large percentage differences in duplicate tests were due to actual changes in residual air value through shift in pulmonary mid-position rather than to technical error. These points have been discussed in the third paper of this series (2), and will be considered again below.

The 158 duplicate determinations of residual air may be grouped according to the clinical conditions of the subjects, as follows:

- 17 on normal subjects;
- 22 on 22 patients with chronic cardiac disease in varying degrees of cardiac insufficiency;
- 48 on 46 patients with chronic pulmonary tuberculosis, including many with pneumothorax or thoracoplasty;
- 49 on 36 patients with pulmonary fibrosis or emphysema;
- 22 on 22 patients with miscellaneous diseases of the chest—bronchiectasis, carcinoma of the bronchus, chest deformity, and pneumonectomy.

Figure 1 summarizes the residual air/total capacity ratio found in these five groups. The increased ratio in the emphysema group was comparable to that reported in the literature by other methods (4, 5, 6). The ratio in our cardiac cases, however, showed less of an increase above normal than has been reported by other methods (7, 8, 9, 10). The 8 per cent increase of the residual air/total capacity ratio above the normal levels, in this cardiac group, is about the same as the

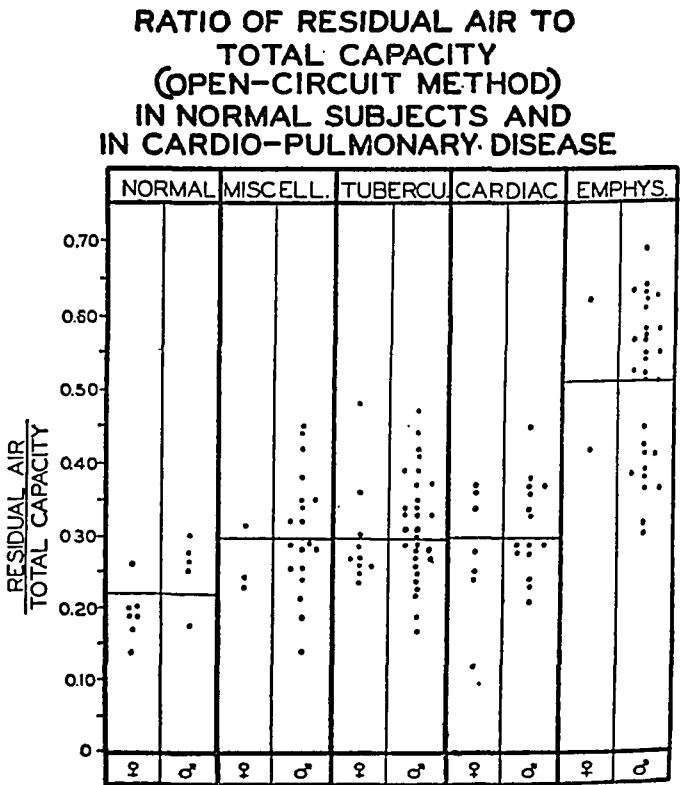


FIG. 1. RESIDUAL AIR/TOTAL CAPACITY RATIO IN INDIVIDUAL CASES IN VARIOUS CLINICAL GROUPS (1) Normal subjects, (2) miscellaneous chest diseases, (3) tuberculosis, (4) cardiac disease, (5) pulmonary emphysema.

relative increase found in the tuberculous and miscellaneous groups where there has been a shrinking of the vital capacity. A further analysis of the residual air in various types and degrees of cardiac insufficiency will be presented in a subsequent paper.

*Relative constancy of the alveolar air sample taken at the start, before the beginning of pure oxygen breathing: elimination of this measurement from procedure*

In Figure 2 are charted all the values for alveolar nitrogen percentage taken at rest under basal conditions while the patient breathed atmospheric air, i.e., before the beginning of pure oxygen breathing. This is the "alveolar  $\bar{a}$ " sample of our earlier papers. From the data illustrated in Figure 2, it is found that 93 per cent of the alveolar  $\bar{a}$  values lie between 79.50 and 82.49 per cent, with a mean value approximately 81.0 per cent. Thus, by reason of the large (alv.  $\bar{a}$  N<sub>2</sub> — alv.  $\bar{p}$  N<sub>2</sub>) difference in the open circuit method (see formula above), the assumption of 81.0 per cent as a constant for alv.  $\bar{a}$  in this formula is unlikely to lead to any important error in the final value of functional residual air. We have recalculated (Figure 3) the data of the 316 residual

air determinations, using 81.0 per cent as a constant value for alveolar  $\bar{a}$  nitrogen, and have found this presumption to be correct. Only 5 per cent of cases showed an alteration of more than 50 cc. in functional residual air value as a result of this recalculation. In all of these the functional residual air was over 2500 cc.; thus the percentage error here was small. In only 2 cases was this alteration over 85 cc. (one a difference of 101 cc., the other 104 cc.). One of these latter cases was an extreme emphysema, the other a combined emphysema and cardiac case.

Thus it seems fair to conclude that the alveolar  $\bar{a}$  sampling can be omitted from the open circuit technique. This shortens and simplifies the procedure. A detailed description of the revised open circuit technique is given at the end of the paper.

*Alveolar air sample taken at the end of the period of pure oxygen breathing: its usefulness as a measure of pulmonary emptying rate, and the physiological significance of this function*

According to the open circuit procedure, the subject, under basal conditions, breathes pure oxygen for 7 minutes, thus washing out the nitrogen in the pulmonary air spaces. This expired air is

### ALVEOLAR NITROGEN CONCENTRATION (%), ROOM AIR BREATHING

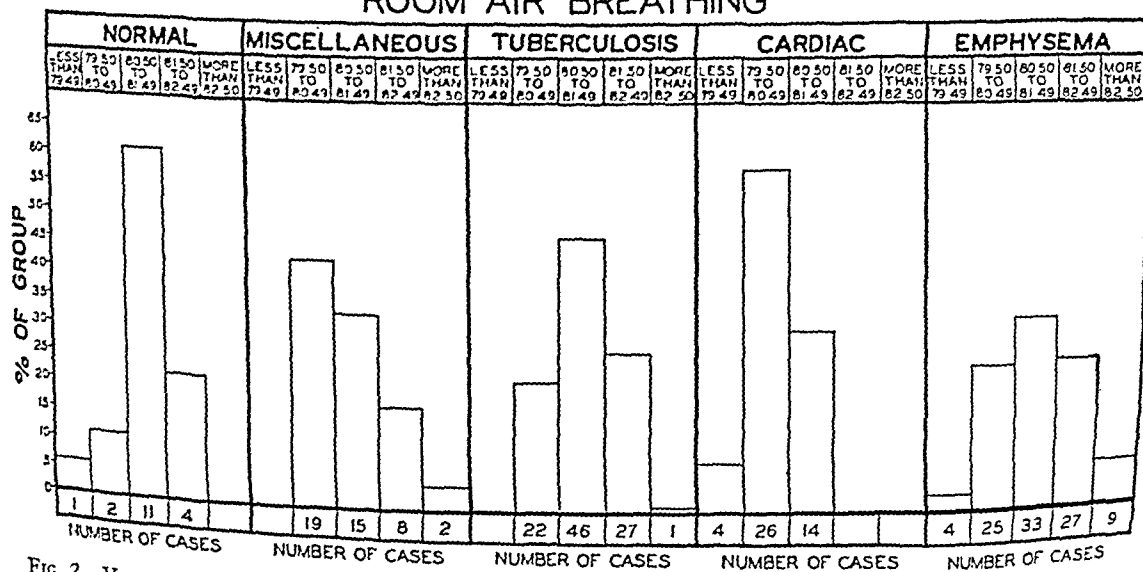


FIG. 2. VALUES OF ALVEOLAR NITROGEN CONCENTRATION, WITH THE SUBJECTS BREATHING ATMOSPHERIC AIR, IN VARIOUS CLINICAL CONDITIONS

# **ERROR IN FUNCTIONAL RESIDUAL AIR DUE TO THE ASSUMPTION OF 81.00 % AS THE STANDARD VALUE FOR ALVEOLAR $\bar{A}$**

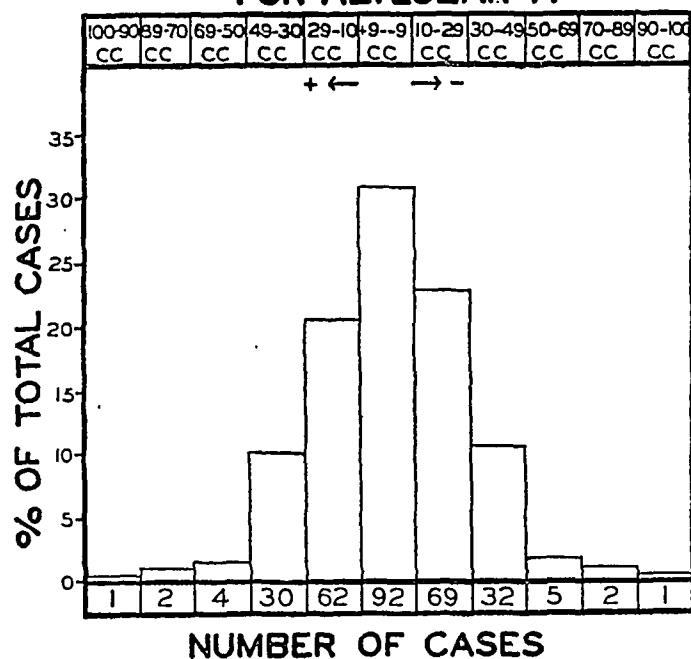


FIG. 3. THE EXTENT OF ERROR IN THE VALUE OF RESIDUAL AIR (OPEN CIRCUIT METHOD) CAUSED BY ASSUMING 81.0 PER CENT AS THE NORMAL ALVEOLAR NITROGEN CONCENTRATION, AS COMPARED WITH RESIDUAL AIR VALUES CALCULATED FROM ALVEOLAR NITROGEN CONCENTRATIONS MEASURED BY EXPERIMENT

collected and subsequently analyzed for nitrogen content. At the end of the 7 minutes of oxygen breathing, the subject exhales completely (into a separate circuit) and a sample of expired (alveolar) air is taken into an evacuated sampling tube.

The procedure of washing out the lungs by oxygen breathing, considered from the purely ventilatory standpoint, is almost ideal for the purpose of demonstrating the effectiveness of distribution of tidal air through alveolar spaces during quiet breathing, since it is only through the adequate distribution of inhaled pure oxygen into the pulmonary spaces that the nitrogen contained in them can be displaced and exhaled.

Thus the completeness of the washing-out of nitrogen from the lungs by a stated period of pure oxygen breathing (which we have termed the *pulmonary emptying rate*) can be considered an effective measure of distribution of tidal air through alveolar spaces. In an efficiently ventilated lung, therefore, an alveolar specimen taken at the end of a relatively short period of oxygen

breathing should show a low nitrogen concentration; in an inefficiently ventilated lung, the nitrogen concentration of this specimen should be high.

This concept is corroborated by the analysis of the data covering "alveolar  $\bar{p}$ " nitrogen measurements in 316 residual air determinations, as illustrated in Figure 4.

If one takes as an upper limit an "alveolar  $\bar{p}$ " nitrogen of 2.5 per cent, one finds that 94 per cent of the determinations in normal subjects, and 100 per cent of those on cardiac patients, are well below the level; whereas above it are 17.7 per cent of tuberculous patients, 17 per cent of the miscellaneous group, and 97 per cent of the emphysema patients.

The difference in performance between the normal and the cardiac groups, on the one hand, and the groups with organic pulmonary disorders, on the other, is thus well defined, though perhaps not unexpected.

It will be apparent that pulmonary emptying, or effectiveness of ventilation, depends in great part upon certain obvious anatomical and physiological relationships. A subject, for example, with small functional residual air, whose tidal air is large and respiratory rate rapid, will, other things being equal, tend to empty his pulmonary spaces rapidly. The emptying that will be expected to occur in a given time with a known functional residual air, effective tidal air, and rate of respiration, and assuming perfect intrapulmonary mixing, can be easily calculated by the usual compound interest equation (the interest rate having here a negative value). In most emphysematous subjects, the large ratio of residual air to tidal air is an important factor in delayed emptying. In addition, however, there is the factor of unequal distribution of tidal air, certain large regions of the lungs being often very inadequately ventilated. This factor is more difficult to express quantitatively, but in advanced pulmonary fibrosis and emphysema is usually the dominant influence in producing delayed pulmonary emptying. A striking evidence of this tendency to inadequate ventilation is shown in Figure 4, where practically all cases of pulmonary fibrosis and emphysema show a decreased rate of pulmonary emptying, including those subjects with relatively small residual airs as well as those with large.

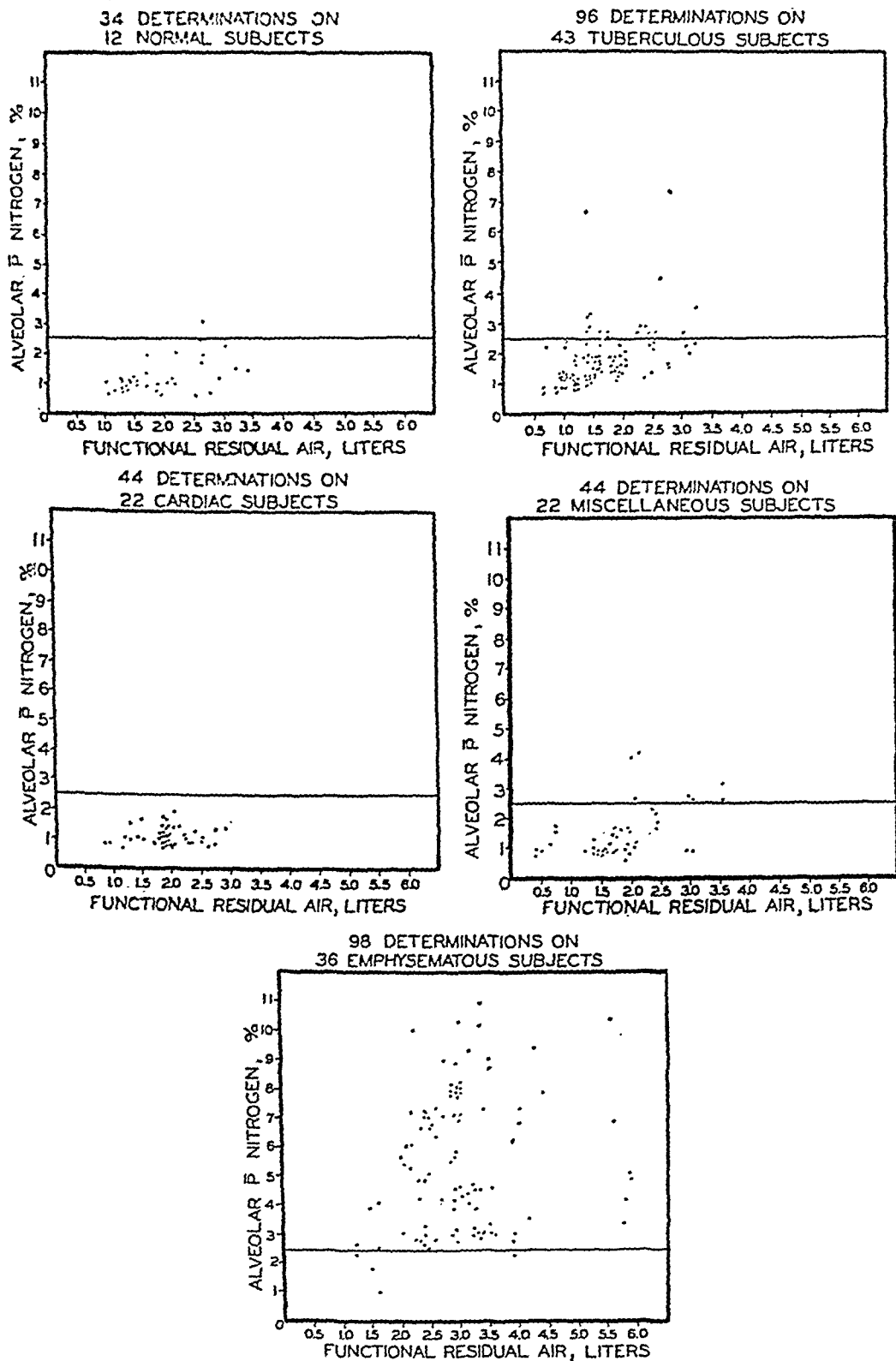


FIG. 4. "PULMONARY EMPTYING RATE"—ALVEOLAR NITROGEN CONCENTRATION AT THE END OF 7 MINUTES OF PURE OXYGEN BREATHING IN 156 DUPLICATE DETERMINATIONS IN VARIOUS CLINICAL CONDITIONS, AS RELATED TO THE SIZE OF THE FUNCTIONAL RESIDUAL AIR



Arterial oxygen unsaturation, which was also studied in a number of cases (Figure 5), was only partially correlated with pulmonary emptying, low saturation occurring at times with rapid emptying, and normal saturation with slow emptying. This is not unexpected, as there may be all degrees of vascular perfusion of poorly aerated regions of the lungs.

Of considerable interest to us was the consistent performance of the group of cardiac patients in their effective pulmonary emptying. None of these, it is true, had very large residual air values, but it seemed theoretically not unlikely that the "lungenstarre" of pulmonary congestion might interfere with effective aeration of some of the perfused pulmonary air spaces. Such was evidently not the case in any of the patients whom we studied, as far as this particular criterion of pulmonary emptying indicated. Objection might

be raised that the low alveolar nitrogen values were due to poor sampling, since these patients often had small reserve air volumes. The fact, however, that alveolar carbon dioxide levels were comparable in these samples with arterial carbon dioxide tensions eliminates this objection. That hyperventilation, which was definite in most cases, may have played a part in the low alveolar  $\bar{p}$  nitrogen percentages is entirely possible, but this does not alter the fact of effective pulmonary emptying in every case under the conditions of the test. Since some of the cardiac patients had a moderate arterial anoxemia, between 89 and 91 per cent, it is suggested that there were other mechanisms for this than inadequate ventilation of perfused alveolar spaces; *i.e.*, perfusion of regions completely unventilated (shunting) or other factors removing air from immediate contact with blood such as by dilatation and congestion of alveolar

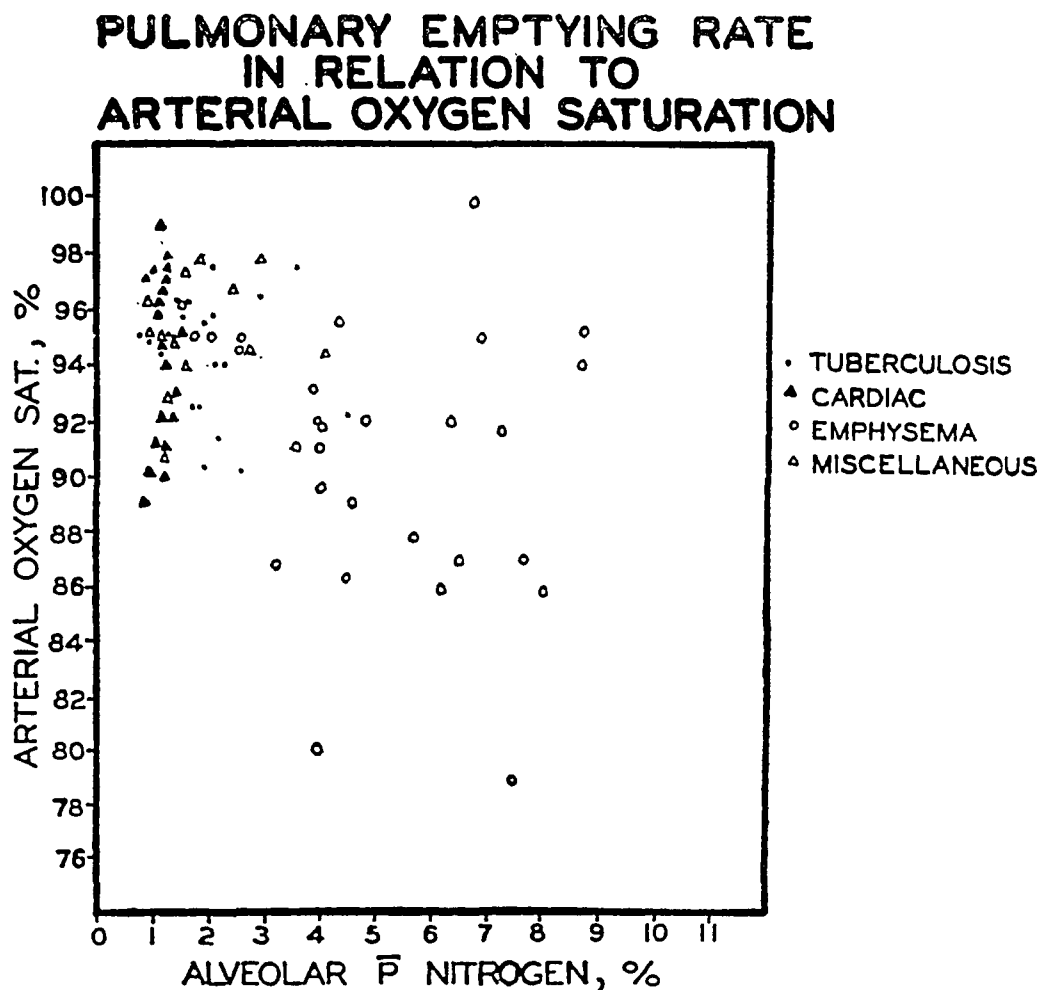


FIG. 5. RELATION BETWEEN ARTERIAL OXYGEN SATURATION AND PULMONARY EMPTYING RATE IN VARIOUS CLINICAL CONDITIONS

"Miscellaneous" refers to a group of miscellaneous chest conditions (see text).

capillaries, or edema, or thickened, less permeable alveolo-capillary walls—in other words, defective diffusion of gases through fluids, as distinct from defective distribution of gases through air spaces.

At this point it should probably be emphasized that this particular test of "pulmonary emptying"—the alveolar nitrogen after 7 minutes of oxygen breathing—is in certain respects quite arbitrary. It tends, as a matter of fact, to demonstrate particularly defects in emptying under conditions found in pulmonary emphysema with relatively large residual air values. Under other conditions, a 3-minute or even a 1-minute period of oxygen breathing will more readily demonstrate slight degrees of defective intrapulmonary mixing. Also, under certain conditions, the small amount of nitrogen excreted from the body during oxygen breathing becomes a significant factor in the alveolar  $\bar{p}$  value. An exhaustive analysis of this question has been undertaken by one of the present authors (R. C. D.) and will be reported separately. It is probably fair, however, to consider the pulmonary emptying rate, above described, as an approximate clinical index of effectiveness of ventilation. As an index of pathological intrapulmonary mixing, the pulmonary emptying rate has to be considered along with other factors—functional residual air, tidal air, and rate of respiration.

#### *"Anatomical" versus "physiological" residual air volumes*

Reference to Figure 4 shows that, in a considerable number of cases of advanced emphysema, the alveolar  $\bar{p}$  nitrogen percentage is very high, up to 8 or 10 per cent or even higher. In other words, 7 minutes of oxygen breathing has not been able to remove more than 90 per cent of the alveolar air originally present in the lungs. The question naturally arises whether in such instances there may not be stagnant or very poorly ventilated lung spaces which remain high in nitrogen after oxygen breathing, but whose contents cannot be adequately exhaled into an "alveolar" air sample, the true mean alveolar nitrogen being actually higher than that obtained. The value obtained for residual air in these cases would

therefore be erroneous. As pointed out in the third paper of this series (2), this excessive stagnation of intrapulmonary air can sometimes be proved by prolonging the time of pure oxygen breathing to 10 or 12 minutes, and a more accurate residual air determination can be obtained in this way.

The ultimate in pulmonary stagnation probably occurs in certain cases of large positive-pressure bulla formation. There is one such case in the present series, a man of 40 with an expanding bulla occupying two-thirds of the left thoracic cavity and displacing the mediastinum to the right. Residual air determination by the open circuit method revealed a very small functional residual air volume, and a rapid and complete pulmonary emptying with low alveolar  $\bar{p}$  nitrogen. The interpretation of these findings is clear: the large bulla was inactive in pulmonary function; in fact completely isolated from the working pulmonary mechanism, except for the small increment of inspiratory air that must have been drawn into the bulla in order to maintain its positive pressure. This case, incidentally, is the only one in the emphysema group that showed a pulmonary emptying rate appreciably below 2.5 per cent (Figure 4).

The distinction between physiologically ventilating, pathologically ventilating, and non-ventilating pulmonary air spaces, is an interesting one. In the last named of these, the separation between physiological and anatomical residual air volumes becomes practically complete.

The open circuit method of residual air measurement, when combined with the alveolar  $\bar{p}$  nitrogen, or pulmonary emptying rate, thus provides more than a value for static or anatomical residual lung volume. It furnishes an index of mixing, or effective distribution, of tidal air through the alveolar spaces during quiet breathing. When these measurements are included with the values for the other divisions of lung volume and with those of total resting pulmonary ventilation and respiratory rate, the purely ventilatory aspect of pulmonary function at rest can be analyzed with a fair degree of completeness. We have discussed elsewhere (11) the dual classification of pulmonary function into ventilatory (air displacement) and respiratory (respiratory gas exchange).

*Revised technique of open circuit method for residual air*<sup>2</sup>

The apparatus used<sup>3</sup> has been described in the third paper of this series (2). It consists essentially of three parts: (a) a standard 100-liter Tissot gasometer; (b) an open circuit, with the inspiratory tubing leading from an anesthesia bag (which in turn is kept partly filled with oxygen from a tank) to the mouthpiece, and the expiratory tubing from mouthpiece to gasometer; (c) a second open circuit, to which the subject can be connected by turning the respiratory valve at the mouthpiece, with expiratory tubing leading out past evacuated sampling tubes for alveolar air sampling.

One change has been made in the apparatus: A long vertical drum revolving at constant rate has been attached to the side of the Tissot gasometer. A pen attached to the counterweight of the spirometer records on this drum each expiratory movement, thus affording a measure of respiratory rate and expiratory amplitude.

*Procedure.* In a preliminary test, the external lung volumes—tidal air, vital capacity, complemental and reserve air—are recorded on a spiograph. This has the added advantage of training the subject in breathing procedures. After a 15-minute rest period, the subject, under basal conditions and lying supine except for a two- or three-pillow head and shoulder elevation, is attached to the mouthpiece, with the valve turned to the second circuit so that he is inhaling outside air. (The main circuit and gasometer have previously been thoroughly washed with oxygen by 6 successive fillings and emptyings of the Tissot gasometer with 15 to 20 liters of oxygen through the entrance bag.) With the gasometer empty, volume and temperature readings are taken. Oxygen flow at approximately the (volume) rate of the subject's resting ventilation is then started from the tank. Precisely at the subject's expiratory level, just before inspiration begins, the valve is turned to shift the subject's breathing into the oxygen circuit. The subject then breathes oxygen quietly for 7 minutes. (At a convenient time during this interval, the second of the respiratory valves at the mouthpiece is turned, thus shutting off the inspiratory tubing of the second [atmospheric air] circuit. This permits an uncontaminated alveolar sampling later. In subjects with a small reserve air, such as cardiac patients, it is practical to wash out the second circuit with oxygen during this interval.) At the end

of the inspiratory phase nearest the exact moment of termination of the 7-minute period, the valve is turned, shifting the subject from first to second circuit, and he is instructed to exhale completely, thus furnishing the alveolar  $\bar{p}$  sample which is taken in the evacuated sampling tube. The subject may then be disconnected from the apparatus. The reason the valve is turned at peak of inspiration is to provide a larger volume of air for washing out the second circuit for the alveolar sample. The amount of nitrogen contained in this tidal air is negligible, so that its loss from the total expired air in the gasometer will cause no error. Finally, the main circuit is flushed with about 10 liters of oxygen into the gasometer, so as to wash any remaining nitrogen out of the inflow tubing. The second volume and temperature readings on the gasometer are recorded, and the outflow tubing is washed through promptly with the air from the gasometer. Two samples are taken from the gasometer for analysis.

The gasometer and main circuit are washed out with oxygen, as before; 15 to 30 minutes are allowed for the subject's lungs to return to conditions of room air breathing; and the determination is then repeated.

Gas analyses are all now carried out by the use of the Van Slyke-Neill apparatus. Certain minor alterations in the procedure as described by Peters and Van Slyke (12) have been kindly communicated to us by Dr. N. L. Kaltreider.

The calculation of functional residual air is as follows:

$$F.R.A. \text{ (dry)} = \frac{(V + DS)(NS - NO)}{81 - \text{alv. } N} - C.$$

*F.R.A. (dry)* = functional residual air in cc. (calculated as dry gas).

*V* = volume reading of spirometer gas, corrected to 37° (dry).

*DS* = volume of dead space under spirometer, corrected to 37°.

*NS* = per cent nitrogen in spirometer.

*NO* = per cent nitrogen in oxygen from tank (usually 0.0 to 0.4 per cent).

*Alv N* = per cent nitrogen in alveolar air at end of oxygen breathing.

*C* = correction for nitrogen excreted from body during period of oxygen breathing, expressed in cc. This factor, previously taken by other investigators and by the present authors as a flat correction for all subjects, has more recently been found by Cournand and Riley (13) to vary with bodily size according to the linear relation  $C = ((B.S. \times 96.5) + 35) \div 0.80$ , where *B.S.* = body surface in square meters.

The final figure for functional residual air volume is computed from *F.R.A. (dry)* by correcting for water vapor saturation, as exists (approximately) within the lungs.

$$F.R.A. = F.R.A. \text{ (dry)} \times \frac{\text{Barometer pressure}}{\text{Barometer pressure} - 48}$$

## SUMMARY

1. The nitrogen concentration of a sample of alveolar air taken at the end of a period of pure oxygen breathing provides an index of pulmonary

<sup>2</sup> A footnote in the third paper of this series indicated that the authors were planning a simplification of the method in which no alveolar samples would be necessary. While such a method could be used with approximate accuracy in almost all cases, we have retained the "alveolar  $\bar{p}$ " nitrogen measurement because of the importance and independent significance which this measurement affords.

<sup>3</sup> The apparatus was made by the Warren E. Collins Company, Boston, Massachusetts. The special 5-way respiratory valve was designed and constructed for us by Mr. Herman P. Roth of the Warren E. Collins Company.

emptying which is useful as a measure of effectiveness of ventilation.

2. This procedure detects delayed pulmonary emptying, particularly in cases of pulmonary fibrosis and emphysema. Patients with such conditions have alveolar nitrogen concentrations, after 7 minutes of oxygen breathing, above 2.5 per cent; normal subjects and patients with chronic heart disease have values below 2.5 per cent.

3. One hundred and fifty-eight determinations of residual air by the open circuit method are presented and analyzed.

4. A revised and somewhat simplified method for open circuit determination of residual air, with simultaneous measurement of pulmonary emptying, is described.

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# A COMPARISON BETWEEN DEHYDRATION FROM SALT LOSS AND FROM WATER DEPRIVATION

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In 1935 Kerpel-Fronius (1) demonstrated in rabbits that there are two distinct types of dehydration, depending upon whether the condition is associated with (1) a primary loss of salt or (2) a shortage of water not accompanied by a corresponding loss of salt. He pointed out that the former is characterized by circulatory disturbances, while the latter is characterized by thirst. In a later paper (2) Kerpel-Fronius referred to the two conditions as "Durstexsikkose und Salz-mangelexsikkose" and emphasized the fact that in animals dehydrated by loss of salt the principal loss of fluid is from the extracellular portion, including the blood, whereas in thirsting animals the water loss is distributed among all fluid compartments of the body, including the large intracellular portion.

It is our impression that most physicians, including ourselves, have not been sufficiently aware of this important distinction and its therapeutic implications. The present report concerns itself with a study of these two types of dehydration in normal human subjects. The first two experiments (IA and IB) deal with abnormal loss of salt while the water intake is adequate. The other two experiments (IIA and IIB) deal with water deprivation with no abnormal loss of salt.

## PROCEDURE

*Experiment IA. Abnormal loss of salt; water intake adequate.* Subject W. M., a healthy male, aged 26, was weighed and placed upon a salt-poor diet (containing approximately 1 gram of NaCl per day) for 6 days. After this preliminary period a Miller-Abbott tube (3) was passed to the proximal jejunum, the position of the tube was verified by x-ray examination, and constant suction was applied for 4 days. During this 4-day period no food was allowed, but water was administered daily, either orally or in the form of intravenous 5 per cent dextrose in distilled water, in amounts sufficient to insure a normal urinary output. Also, the tube was irrigated frequently with measured amounts of tap water which

were figured in as part of the total oral intake. After removal of the Miller-Abbott tube, the subject was allowed salt-free water orally and intravenously for a period of 24 hours. On the following day he was given 3675 cc. of Ringer's solution (containing 33.1 grams of NaCl) intravenously. On the 2 final days he was allowed water ad libitum and a salt-poor diet.

Each morning after voiding the subject was weighed. Daily measurements were made of the urine volume and of the losses of sodium and chloride in the urine and from the jejunal drainage.<sup>2</sup> Frequent determinations<sup>2</sup> were also made of the hematocrit, plasma proteins, serum sodium, plasma chlorides, plasma carbon dioxide combining power, and of the arterial blood pressure. The subject was watched closely for any signs of dehydration.

*Experiment IB. Abnormal loss of salt; water intake adequate.* Subject E. B., aged 21, was a normal male except for the presence of an orthostatic albuminuria. The procedure was identical to that in Experiment IA with the following exceptions. The Miller-Abbott tube suction was continued for 5 days instead of 4. Distilled water was used in place of tap water throughout the experiment. The salt-poor diet was resumed as soon as the Miller-Abbott tube had been removed. Three thousand cubic centimeters of Ringer's solution (containing 27 grams of NaCl) were administered on the second day following removal of the tube.

*Experiment IIA. Water deprivation; no abnormal loss of salt.* Subject E. B. at the conclusion of Experiment IB was studied further. He continued on a salt-poor diet for 2 days and had water ad libitum. Then the dehydration period began and all fluids were eliminated for 4 days. On the 5th day his mouth was so dry he could not eat, so 600 cc. of distilled water were allowed. During the recovery period distilled water was allowed ad libitum, the diet remaining salt-poor as before. Blood studies and other measurements were carried out as in Experiments IA and IB. Also several determinations were made of the blood non-protein nitrogen.

*Experiment IIB. Water deprivation by means of total*

<sup>2</sup> The chemical determinations were run in duplicate and standard methods were used throughout as follows: Sodium: Butler and Tuthill (4). Potassium: Shohl and Bennett (5). Chloride: Wilson and Ball (6). Nitrogen: Macro-Kjeldahl. Total urine solids: Shackell (7). Carbon dioxide combining power: Van Slyke (8). The plasma protein estimations were based upon plasma specific gravities according to Weech's formula (9), the specific gravity determinations being made by the Barbour and Hamilton falling drop method (10).

<sup>1</sup> Aided by a grant from the Horace H. Rackham School of Graduate Studies.

fasting. This experiment, though somewhat similar to Experiment IIA, differed from it chiefly in that the dehydration period consisted of a total fast. The subject, T. G., a normal male aged 23, after having been placed upon a salt-poor diet for 4 days, was persuaded to go without food or water for 3 days. During the subsequent 3 days he still received no food but was allowed distilled water ad libitum. In fact, he was requested to drink even more than he desired, and on the last of these 3 days (day 10) he was given, in addition, 4.8 grams of potassium as a dilute neutral solution of potassium salts ( $K_2HPO_4$  and  $KH_2PO_4$ ). On the next 2 days (days 11 and 12) he was given a submaintenance diet consisting of 2 liters of whole cow's milk per day. On the 12th day he was given, in addition to the milk, 9 grams of sodium chloride.

Every morning after voiding the subject was carefully weighed on a balance accurate to 2 grams. Daily determinations were made of the urine volume, urine specific gravity, urine solids, and urinary excretions of sodium, potassium, chloride, and nitrogen. The same blood studies were carried out as in the other three experiments. During the difficult fasting period the subject was attended both day and night and seemed to cooperate in every particular.

#### RESULTS

In Experiment IA, the data of which are shown in Table I and Figure 1, the body weight de-

creased by 4.3 kgm. during the 4 days of the Miller-Abbott suction. During this period 8.4 grams of Na and 13.9 grams of Cl, equivalent to about 22 grams of NaCl, were lost from the body, mostly through the tube, only a very small amount being excreted in the urine. The urine output averaged 1430 cc. per day. The hematocrit and plasma protein concentration showed a gradual and continuous increase. The serum Na concentration dropped from 308 to 274 mgm. per cent, a decrease of about 11 per cent, while the plasma Cl concentration fell from 568 to 464 mgm. per cent, a decrease of about 18 per cent. The  $CO_2$  combining power showed little change. Towards the end of the dehydration period, the subject became weak and apathetic and the blood pressure fell to 85 mm. Hg systolic. He became faint on assuming the upright posture and preferred to lie flat in bed without a pillow. There was pronounced anorexia. *There was no thirst.*

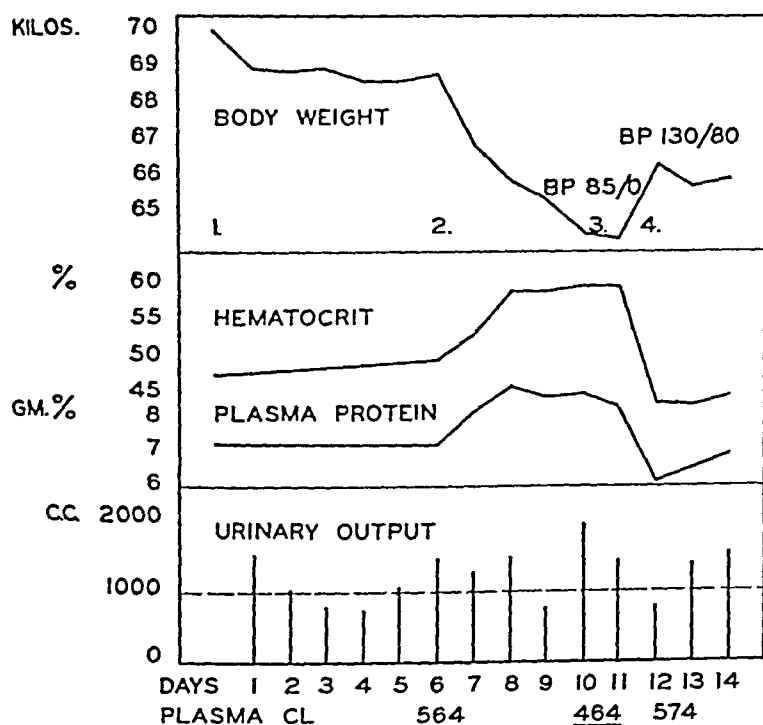
During the 24-hour period immediately after removal of the tube (day 11) the situation was essentially unchanged. The hematocrit and plasma protein concentration remained high while the

TABLE I

*Data on Experiment IA. Subject W. M. Dehydration by means of constant jejunal suction with Miller-Abbott tube; water intake adequate*

Days	Body weight	Fluid intake		Suction			Urine			Blood					Remarks
		Mouth	Intra-venous	Amount	Na	Cl	Amount	Na	Cl	Hemato-crit	Plasma protein	Serum Na	Plasma Cl	Plasma $CO_2$ C.P.	
	kgm.	cc.	cc. 5% dextrose	cc.	grams	grams	cc.	grams	grams	per cent	grams per cent	mgm. per cent	mgm. per cent (as NaCl)	volume per cent	
0	70.1									47.2	7.2	313	566	47	
1	69.1						1520	5.43	8.78						Salt-poor diet.
2	69.0						1050	2.32	3.35						
3	69.2						840	2.61	3.68						
4	68.7						800	2.50	3.59						
5	68.7						1150	0.89	1.44						
6	68.9						1450	0.45	0.86	50.5	7.2	308	568	47	
7	66.5	1500	0	1460	1.95	3.42	1380	0.49	1.22	53.3	8.1	271	553	31	Miller-Abbott tube suction. No diet.
8	66.0	2000	1900	1540	2.40	3.70	1540	0.17	0.12	59.2	8.8	284	512	41	
9	65.4	2750	950	2230	2.14	3.49	840	0.09	0.07	59.3	8.5	288	477	50	
10	64.6	1500	1850	1040	1.05	1.89	1960	0.04	0.11	60.2	8.6	274	479	38	
11	64.3	250	2000				1410	0.02	0.13	60.0	8.2	282	464	47	Salt-free water given.
12	66.4	0	3675*				810	0.40	0.44	42.4	6.1	298	574	41	Ringer's solution given.
13	65.7	2000	0				1370	1.59	1.91	42.2	6.5	308	568	41	Salt-poor diet resumed.
14	65.9	Ad lib.	0				1550	2.00	2.10	44.8	6.8	325	569	45	

\* Ringer's solution.



1. SALT POOR DIET
2. MILLER-ABBOTT TUBE  
NA LOSS 8.4 GM.(21.0)  
CL LOSS 13.9 GM.(23.1)
3. TUBE OUT  
SALT FREE WATER GIVEN
4. RINGER'S SOLUTION  
3675 C.C. I.V.

URINE VOLUMES ADEQUATE

DROP IN BLOOD PRESSURE

NO THIRST

FIG. 1. GRAPHIC PRESENTATION OF EXPERIMENT IA. SUBJECT W. M. DEHYDRATION BY MEANS OF CONSTANT JEJUNAL SUCTION WITH MILLER-ABBOTT TUBE; WATER INTAKE ADEQUATE

serum Na, plasma Cl, and the blood pressure remained low. The weakness, anorexia, apathy, and orthostatic fainting continued.

As soon as the Ringer's solution was administered, on the 12th day, there was a prompt improvement in the subject's condition. The blood became diluted, the serum Na and plasma Cl returned to normal, the blood pressure rose to normal, and the disagreeable symptoms vanished.

At the end of the recovery period, the body weight was about 3 kgm. less than at the beginning of the experiment, undoubtedly the result of 6 days of fasting. The final values for hematocrit and plasma protein were lower than the initial values, probably due to the fact that during the course of the experiment a total of about 400 cc. of blood was taken for the various blood tests.

Experiment IB was essentially the same as IA and yielded similar results. As shown by the data in Table II and Figure 2, there occurred a loss in weight, a rise in hematocrit and a drop in blood pressure. Fainting, weakness, apathy and anorexia were pronounced. *There was no thirst*; in fact, there seemed to be an aversion to water.

It will be noted that the plasma protein values did not rise so noticeably in Experiment IB as in Experiment IA and this might be related to the orthostatic albuminuria of the IB subject. The administration of Ringer's solution restored the subject to normal.

The results in Experiments IIA and IIB stand out in marked contrast to those in IA and IB. In Experiment IIA there was no abnormal loss of salt and dehydration was brought about by water deprivation. The data given in Table III and Figure 3 show a loss in body weight of 2.6 kgm. during the period of water deprivation. There was no significant change in hematocrit, serum Na, or plasma  $\text{CO}_2$  combining power. The plasma Cl rose from 554 to 584 mgm. per 100 cc. The daily urinary output averaged 614 cc. The excretion of Na and Cl increased slightly during the period of water deprivation, but remained low nevertheless. The blood non-protein nitrogen rose from 33 to 48 mgm. per cent. *Thirst became acute* and the mouth and throat became very dry. The voice became weak and high in pitch. It will be recalled that on the last day of the dehy-

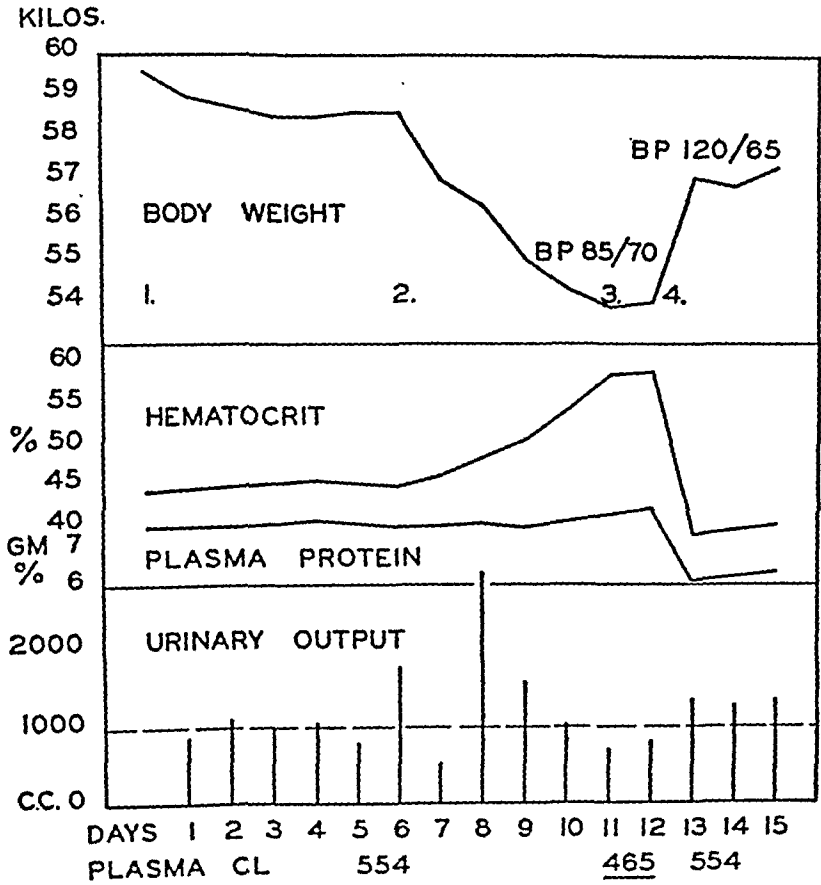


TABLE II

Data on Experiment IB. Subject E. B. Dehydration by means of constant jejunal suction with Miller-Abbott tube; water intake adequate

Days	Body weight	Fluid intake		Suction			Urine			Blood					Remarks
		Mouth	Intra-venous	Amount	Na	Cl	Amount	Na	Cl	Hem-ato-crit	Plasma protein	Serum Na	Plasma Cl	Plasma CO <sub>2</sub> C.P.	
	kgm.	cc.	cc. 5% dextrose	cc.	grams	grams	cc.	grams	grams	per cent	grams per cent	mgm. per cent	mgm. per cent (as NaCl)	volume per cent	
0	59.7									44.3	7.5	292	574	49	
1	59.0						995	1.87	3.54						Salt-poor diet
2	58.8						1130	0.88	1.68	45.3	7.5	292	561	51	
3	58.6						1000	0.64	1.22						
4	58.5						1110	0.45	1.04	45.6	7.7	297	553	55	
5	58.8						870	0.30	0.56						
6	58.7						1170	0.35	0.48	44.6	7.5	302	554	58	
7	57.0	4000	0	4300	2.18	4.59	540	0.10	0.18	46.6	7.5	301	531	51	Miller-Abbott tube suction. No diet.
8	56.3	2600	2000	1090	1.47	2.28	2980	0.16	0.16	48.0	7.6	291	526	53	
9	55.4	3200	0	1730	2.16	3.39	1580	0.06	0.08	51.7	7.5	299	507	50	
10	54.3	2500	0	1560	1.87	3.06	1080	0.01	0.06	53.8	7.7	274	484	48	
11	53.9	2500	0	1270	1.00	1.69	750	0.04	0.02	57.1	7.8	270	467	51	
12	54.0		0				780	0.005	0.04	57.0	7.9	269	465	61	Salt-poor diet resumed.
13	57.0		3000*				1430	0.03	0.23	39.0	6.0	307	554	57	Ringer's solution given.
14	56.9						1310	0.14	0.35						Salt-poor diet continued.
15	57.3						1360	0.45	0.54	40.0	6.4	299	553	55	

\* Ringer's solution.



- 1. SALT POOR DIET
- 2. MILLER-ABBOTT TUBE  
NA LOSS 9.0 GM. (22.5)  
CL LOSS 15.6 GM. (25.7)
- 3. TUBE OUT  
SALT FREE WATER GIVEN
- 4. RINGER'S SOLUTION  
3000 C.C. I.V.

URINE VOLUMES ADEQUATE  
DROP IN BLOOD PRESSURE  
NO THIRST

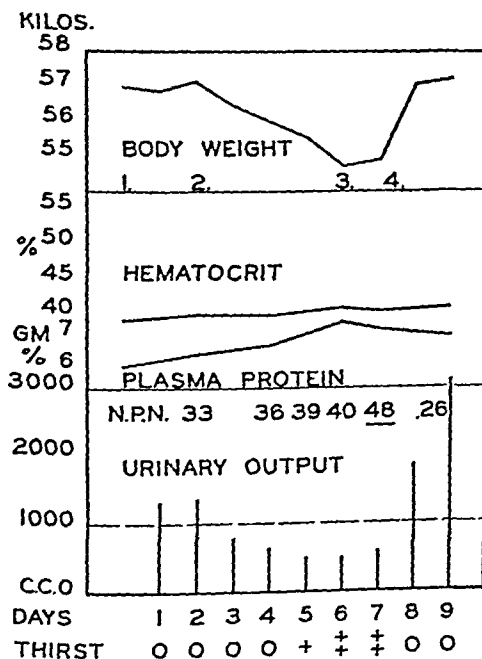
FIG. 2. GRAPHIC PRESENTATION OF EXPERIMENT IB. SUBJECT E. B. DEHYDRATION BY MEANS OF CONSTANT JEJUNAL SUCTION WITH MILLER-ABBOTT TUBE; WATER INTAKE ADEQUATE

TABLE III

Data on Experiment IIA. Subject E. B. Dehydration by means of water deprivation; no abnormal loss of salt

Days	Body weight	Fluid intake (oral)	Urine			Blood					Remarks
			Amount	Na	Cl	Hema-tocrit	Plasma protein	Serum Na	Plasma Cl	Plasma CO <sub>2</sub> C.P.NPN	
	kgm.	cc.	cc.	grams	grams	per cent	grams per cent	mgm. per cent	mgm. per cent (as NaCl)	volume per cent	mgm. per cent
0	57.0					39.0	6.0	307	554	57	
1	56.9	ad lib.	1310	0.14	0.35						
2	57.3	ad lib.	1360	0.45	0.54	40.0	6.4	299	553	55	33
3	56.5	0	770	0.88	0.49						
4	56.0	0	680	1.06	1.36	40.3	6.6	301	571	57	36
5	55.7	0									
6	54.7	0	1020*	1.30*	2.51*	41.7	7.4	298	584	51	40
7	54.9	600	600	0.58	0.87	41.3	7.1	292	584	52	48
8	57.1	3000 + meals	1850	0.28	0.41						
9	57.4	2000 + meals	3180	0.15	0.46	41.7	6.9	279	556	54	26

\* Represents 48-hour period, because of failure to collect urine specimen at proper time.



1. SALT POOR DIET
2. DRY DIET
3. 600 C.C. OF DISTILLED WATER ALLOWED
4. DISTILLED WATER AS DESIRED

OLIGURIA  
MARKED THIRST  
RISE IN N.P.N.

FIG. 3. GRAPHIC PRESENTATION OF EXPERIMENT IIA. SUBJECT E. B. DEHYDRATION BY MEANS OF WATER DEPRIVATION; NO ABNORMAL LOSS OF SALT

dration period 600 cc. of distilled water were allowed so that the subject could eat his food, yet on this day the urinary output was only 600 cc. and the blood non-protein nitrogen continued to rise.

As soon as the subject was allowed distilled

water ad libitum, recovery was prompt. The body weight rose to its previous level, the urine volume increased and the blood non-protein nitrogen returned to normal. The thirst disappeared promptly.

In the last experiment, IIB, dehydration re-

sulted from deprivation of both food and water and the results were quite similar to those in Experiment IIA. The data in Table IV and Figure 4 show that there occurred a loss of weight, oliguria, azotemia, *marked thirst*, and very little change in composition of the blood. There was no significant alteration in hematocrit or in plasma protein concentration. As in the previous experiment, the administration of distilled water promptly alleviated the thirst and was followed by a diuresis and a return to normal of the blood non-protein nitrogen. On the first day of the recovery period, the subject was given 4318 grams of water. The first half of this was taken eagerly within about an hour, but the second half was drunk reluctantly and in small amounts throughout the day. The urine volume for that day was 2090 cc. and the gain in weight was 1257 grams. On the following day 4000 grams of water were offered, but only 3515 grams were drunk, the subject having to force down most of this. The

urinary output for that day was 3110 cc. and there was a loss of 449 grams of body weight.

On the 10th day potassium salts were given and produced no significant changes. The subject continued to lose weight until the final day of the experiment, when 9 grams of NaCl were administered, which resulted in a weight gain of 1070 grams.

In Table IV are shown also other data of interest, such as the specific gravity of the urine and the urinary excretion of nitrogen, sodium, potassium, chloride, and total solids. The specific gravity was high during the 3 days of the dehydration period, being above 1.036 as compared to 1.020 or less on all other days. The excretion of nitrogen averaged about 13 grams during the latter part of the fast and the high value of 16.7 grams occurred with the diuresis on the first day of water administration, the day on which the blood non-protein nitrogen returned to normal. The sodium excretion diminished dur-

TABLE IV

*Data on Experiment IIB. Subject T. G. Dehydration by means of total fasting; no abnormal loss of salt*

Days	Body weight grams	Water drunk grams	Diet		Urine									Blood								Remarks
			Type	Amount grams	Volume cc.	Weight grams	Solids grams	H <sub>2</sub> O grams	Specific gravity	N grams	Na grams	K grams	Cl grams	Stools grams	Weight grams	Hematocrit per cent	Plasma protein grams per cent	Serum Na mgm. per cent	Plasma Cl mgm. per cent	Plasma CO <sub>2</sub> C. P. vol- ume per cent	NPN mgm. per cent	
0	74596														40	47.8	7.7	292	578	48		
1	73949	ad lib.	salt poor	ad lib.	1520	1549	47.8	1501	1.019	13.6	2.23	2.78	3.48									
2	74031	ad lib.	salt poor	ad lib.	1535	1566	58.6	1507	1.020	17.0	0.84	2.53	1.53		30		7.4	279	576	46		
3	74001	ad lib.	salt poor	ad lib.	1120	1148	61.0	1077	1.020	17.5	0.46	2.90	1.00		5	48.6	7.9					Preliminary period. Salt-poor diet.
4	73901	ad lib.	salt poor	ad lib.	1330	1355	58.0	1297	1.019	16.8	0.24	2.58	0.76		45	48.4	7.5	301	576	48	39	
5	72504	0	0	0	430	445	36.2	409	1.036	10.2	0.13	2.10	0.65	0	40	49.7	8.0	301	574	48	40	Total fast. No food or water.
6	71106	0	0	0	450	467	39.5	428	1.037	11.3	0.34	2.02	0.70	0	40	51.6	8.1	298	584	49	42	
7	69777	0	0	0	445	462	37.6	424	1.038	11.2	0.48	1.74	0.67	0	40	49.2	7.7	309	597	31	49	
8	71034	4318	0	0	2090	2117	44.5	2072	1.013	16.7	0.33	1.62	0.32	0	40	50.3	7.7	300	561	47	31	Distilled water ad libitum.
9	70585	3510	0	0	3110	3132	30.2	3102	1.007	12.9	0.21	1.80	0.30	0	35	49.0	7.7	303	556	43		
10	70325	3295*	0	0	2500	2527	46.8	2480	1.011	13.2	0.36	3.06	0.33	175	30	50.1	8.1	289	545	40		Potassium salts given.
11	70042	2143	whole milk	2074	2550	3576	43.0	3535	1.005	15.3	0.38	1.76	0.39	0	30	47.7	7.5	303	563	45		Milk 2074 grams.
12	71112	1687†	whole milk	2083	1880	1899	35.0	1864	1.010	10.4	0.26	2.11	0.72	0	30	45.1	7.4		576	45		Milk 2083 grams. NaCl 9.0 grams.

\* Given with 4.8 grams of K as a mixture of K<sub>2</sub>HPO<sub>4</sub> and KH<sub>2</sub>PO<sub>4</sub>.

† Given with 9 grams of NaCl.

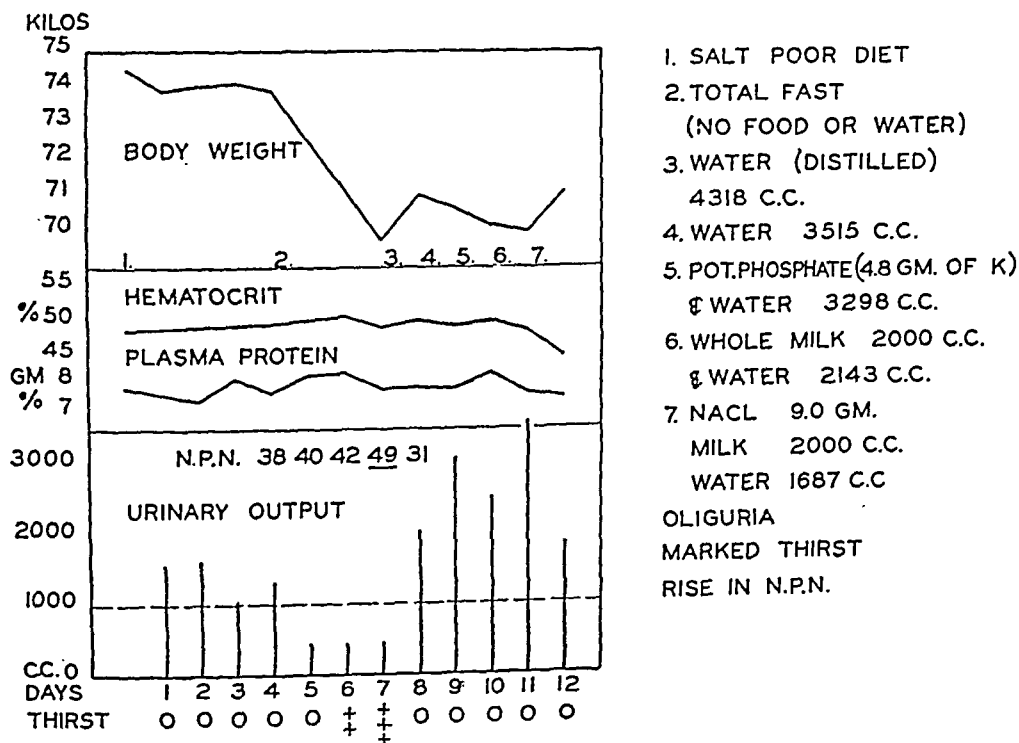


FIG. 4. GRAPHIC PRESENTATION OF EXPERIMENT IIB. SUBJECT T. G. DEHYDRATION BY MEANS OF TOTAL FASTING; NO ABNORMAL LOSS OF SALT

ing the preliminary period with the salt-poor diet and remained at a low level throughout, though there was a slight rise at the time of maximum dehydration. The chloride excretion paralleled in a general way the sodium excretion. During the fast, potassium excretion remained close to the average of 1.9 grams per day except on the day the potassium salts were administered, when it rose to 3.06 grams. The excretion of urinary solids averaged 39.1 grams per day during the fasting period.

Calculations of water and energy balance in Experiment IIB were made according to methods recommended by Newburgh (11), and are summarized in Table V. The values for utilization of protein are derived from the values for nitrogen excretion, a suitable correction being made for the days of water deprivation while nitrogen was being retained and for the day immediately afterward, when the retained nitrogen was being washed out. The values for carbohydrate utilization during the initial days of fasting are estimates based upon other studies of fasting human beings (12, 13). During the last 2 days of the

experiment it was assumed that no glycogen was stored inasmuch as the diet was submaintenance.

The values for water balance are shown in the last column of Table V. There was a negative balance of 2088 grams during the period of dehydration, and a positive balance of 1800 grams on the first day of recovery, when water was allowed ad libitum. The only other large change in water balance was on the final day, when 9 grams of NaCl were ingested, which resulted in a positive balance of 1064 grams.

In order to compare the water balance with the balances of sodium and potassium, some additional data are presented in Table VI. This table includes the 3 days of water deprivation, together with the 2 following days, during which nothing but distilled water was administered. During these 5 days all balances, except for water, were necessarily negative. In columns 2 and 3 are given the balances for Na and K respectively, expressed in milliequivalents. The figures in column 4 refer to the amount of extracellular water which would ordinarily correspond to the sodium balance for the period in question. The figures

TABLE V

Further data from Experiment IIB. Subject T. G. Energy and water balance during total fast and during recovery period

Days	Change in body weight	I. L.	Metabolic mixture			Cal-ories	Water										Remarks
			CHO	P (as N)	Fat		H <sub>2</sub> O drunk	H <sub>2</sub> O of food	Oxi-dation	Pre-formed	I. W.	Urine	Blood	Total in	Total out	Balance	
	grams	grams	grams	grams	grams		grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	
5	-1397	912	100	11.0	142	2025	0	0	241	520	873	409	40	761	1322	- 561	Total fast.
6	-1398	891	50	12.4	160	2022	0	0	234	395	871	428	40	629	1339	- 710	
7	-1329	827	0	13.0	170	1926	0	0	216	261	830	424	40	477	1294	- 817	
																-2088	
8	+1257	904	0	13.0	189	2107	4318	0	236	262	904	2072	40	4816	3016	+1800	Distilled H <sub>2</sub> O ad libitum. No diet.
9	- 449	797	0	12.9	163	1853	3515	0	207	258	799	3102	35	3980	3936	+ 44	
																+1844	
10	- 260	826	0	13.2	169	1922	3298	0	215	264	828	2480	30	3777	3496	+ 281	Potassium salts administered.
11	- 289	892	104	15.3	121	1958	2143	1804	232	86	846	3535	30	4265	4411	- 146	Milk 2074 grams. Milk 2083 grams. NaCl 9.0 grams.
12	+1070	780	104	10.4	108	1710	1687	1812	205	- 9	737	1864	30	3695	2631	+1064	
																+ 918	
Total	-2495									2037			285	22400	21445	+ 955	

TABLE VI

Further data from Experiment IIB. Subject T. G. Relationship of water balance to balance of Na and K during the fasting period

Column 1	2	3	4	5	6	7	8	9	Remarks
Days	Na balance	K balance	Extracellular water corresponding to Na $\left(\frac{\text{Na}}{0.148}\right)^*$	Intracellular water corresponding to K $\left(\frac{\text{K}-0.02 \text{ Na}}{0.14}\right)^*$	"Pre-formed" water	Balance of "available" water	Absolute water balance (Column 7 minus Column 6)	Water losses not accounted for by losses of Na and K (Column 8 minus Columns 4 and 5)	
	m. eq.	m. eq.	grams	grams	grams	grams	grams	grams	
5	- 5.6	- 53.8	- 38	- 384	520	- 561	-1081	- 659	Total fast.
6	-14.6	- 51.6	- 98	- 367	395	- 710	-1105	- 640	
7	-21.0	- 44.7	-142	- 316	261	- 817	-1078	- 620	
	-41.2	-150.2	-278	-1067	1176	-2088	-3264	-1919	
8	-14.4	- 41.4	- 97	- 294	262	+1800	+1538	+1929	Distilled water by mouth ad libitum. No diet.
9	- 9.0	- 46.1	- 61	- 328	258	+ 44	- 214	- 175	
	-23.4	- 87.5	-158	- 622	520	+1844	+1324	+1754	
Total	-64.6	-237.7	-436	-1689	1696	- 244	-1940	- 165	

\* See text for explanation of formulae.

in column 5 refer to the amount of intracellular water which would ordinarily correspond to the potassium balance for the period in question, a correction being made in each case for the potassium present in extracellular fluid. These figures in columns 4 and 5 are derived from those in columns 2 and 3 by applying the formulae indicated in the table. The formulae are those recommended by Peters (14), except that they have been modified to conform with more recent analyses of extracellular fluid as suggested by Gamble

(15). It is obvious that in this type of experiment the values in columns 4 and 5 cannot possibly express actual changes in water balance of intracellular or extracellular water for the simple reason that these values during the 5 days covered by the table are all necessarily negative, even on the day when the water balance was positive. The values for "preformed water" have been carried over from the preceding table and placed in column 6. The figures in column 7 are carried over from the last column of Table V. Columns

8 and 9 are self-explanatory and will be discussed later.

## DISCUSSION

### 1. *The two types of dehydration*

The results of these experiments in human beings substantiate the findings of Kerpel-Fronius in rabbits (1). It appears that the two types of dehydration in question differ from each other not only in mechanism of production but also in symptomatology and in the treatment indicated. In fact, about the only similarity between the two conditions is that implied by the term "dehydration."

The body is continually losing water insensibly by evaporation, frequently more than 1000 or 1200 grams daily. This insensible water ordinarily contains a negligible amount of salt when sweating is avoided (16) and can, therefore, be considered essentially a loss of distilled water. If this loss plus the losses in urine and feces are not replaced by the water of food and drink plus the water available from other sources (water of oxidation and "preformed water"), a condition of true dehydration results, with thirst, oliguria, and a rising blood non-protein nitrogen. There occurs a water shortage in all parts of the body, intracellular as well as extracellular. For if such dehydration were assumed to be localized, let us say, to the extracellular portion of the body, we would then have a hypertonicity of the extracellular fluid as compared to intracellular fluid. The laws of membrane equilibrium demand that the diffusible particles shift until osmotic equilibrium is reached. But since the cell wall is not freely permeable to the most important ions,  $\text{Na}^+$  and  $\text{K}^+$ , this can only mean that water must pass out of the cells until equilibrium is reached. Thus, in water deprivation we are dealing with a diffuse, and not a localized dehydration. This concept fits in well with the fact that only slight changes were observed in the hematocrit during this type of dehydration (Figures 3 and 4).

In the salt-loss type of dehydration, on the other hand, we have a radically different state of affairs. As long as the water intake is adequate, no thirst occurs. There is no shortage of water as such. The deficiency of sodium, however, results in a reduction in the volume of the extra-

cellular fluid (2, 17, 18), which can be thought of as a localized type of dehydration. If we were to postulate that a large loss of sodium could occur without a resulting loss of extracellular water, we should then be postulating a hypotonicity of the extracellular fluid as compared to the intracellular fluid. Such a system would not be in osmotic equilibrium and, therefore, could not endure. Water would be forced to leave the extracellular spaces and either leave the body or pass into the cells, or both, until equilibrium were reached. The net result, therefore, would be a localized dehydration involving the extracellular fluid. Experimental evidence that this actually occurs has been presented by Darrow and Yannet (18). In Experiments IA and IB there was no thirst, and attempts to replace the depleted extracellular fluid with salt-free water merely resulted in a corresponding increase in urinary output. The manifestations of extracellular desiccation are those of peripheral circulatory failure. The reduced extracellular volume includes a reduced plasma volume as indicated by a rise in the hematocrit (Figures 1 and 2). Such a reduction in blood volume results in apathy, weakness, fainting, anorexia, low blood pressure and, if the condition is allowed to progress far enough, to circulatory collapse. This "electrolyte shock," as we have termed it, may be indistinguishable clinically from shock due to other causes, and is brought to mind by the realization that the patient has been losing appreciable amounts of electrolyte-containing fluid. If the blood pressure does not fall too low, and if the water intake is adequate, the urinary volume may remain normal, as was the case in Experiments IA and IB.

Our results and interpretation of the experiments, here described, fit in very well with the demineralization experiments in dogs described by Darrow and Yannet (18). These authors clearly demonstrated the relationship of salt loss to extracellular dehydration. They noted also that "thirst is not an obligatory accompaniment of dehydration" and that "water intake and urine output may be normal in the presence of dehydration."

An interesting and important practical question is the following: In the extracellular type of dehydration, to what extent is the blood plasma

affected? One often hears the statement that in dehydration the plasma volume is protected by the existence of a large amount of fluid in the interstitial reservoir and that no reduction in plasma volume occurs until dehydration is quite advanced. The results of our experiments do not support this concept. In both of our salt depletion experiments we were impressed by the fact that evidence of hemoconcentration appeared within 24 hours after insertion of the Miller-Abbott tube and increased on each succeeding day until the tube was removed. We did not make actual determinations of plasma volumes in our subjects. However, if one considers the blood volumes of W. M. and E. B. to be 5250 and 4500 cc., respectively (computed as 7.5 per cent of body weight), then, since the initial hematocrits were 47.2 and 44.3 per cent, the estimated plasma volumes would be 2772 cc. for W. M. and 2506 cc. for E. B. If we use the change in hematocrit to indicate the change in plasma volume, we arrive at a reduction of 1101 cc. (or 39 per cent) for W. M. and 1008 cc. (or 40 per cent) for E. B. This would correspond to reductions in blood volume of 21 and 22 per cent, respectively, reductions which are of sufficient magnitude to produce shock (19). These calculations give minimum values, as they are based upon the assumption that the total cell volumes remained constant, which was not the case since a considerable amount of blood was taken for the various blood tests (perhaps 200 to 300 cc. during the period in question).

It seems reasonable to conclude, therefore, that serious depletion of extracellular electrolyte causes a decrease in the volume of extracellular fluid, both interstitial fluid and plasma. This leads eventually to peripheral circulatory failure, but before this stage is reached a progressive hemoconcentration can be observed. Thus, frequent hematocrit and plasma protein determinations are of definite value in connection with the diagnosis and treatment of this type of dehydration, a fact which has been emphasized recently by Scudder (20) and by others (21, 22).

## 2. Mixed type of dehydration

It is very common to encounter patients with both the "salt-loss" and the "water deprivation" type of dehydration existing simultaneously. One

of the most frequent causes of dehydration is vomiting, and this generally gives rise to the mixed type. However, this need not always be the case. If vomiting is slight and anorexia marked, it may lead to severe water deprivation without significant salt loss. On the other hand, if vomiting is marked and at the same time is characterized by remissions during which the patient can drink, there may develop a severe electrolyte loss without water deprivation and without thirst. As a rule, vomiting by itself does not result in circulatory collapse, though it does predispose to this condition. Since the ratio of sodium to chloride is low in vomitus, severe depletion of sodium is not likely to occur early and therefore the presenting symptoms of a vomiting patient are more likely to be those associated with thirst and occasionally alkalosis.

In diarrheal disease accompanied by vomiting, we also are likely to find a mixed picture, depending upon which factors predominate in any given case. With diarrhea or ileostomy drainage the relative loss of fixed base is greater than in vomiting and, therefore, the likelihood of circulatory collapse is greater.

If a patient with mixed dehydration is treated either by physiological saline alone or by glucose solution alone, the dehydration may then become converted into one or the other of the pure types. We have seen this actually happen.

The fact that dehydration so commonly occurs in mixed form is probably responsible for much of the confusion that exists. Most textbooks do not distinguish between the two types of dehydration. We often encounter seemingly conflicting statements concerning the nature and treatment of dehydration when in reality the statements apply to entirely different conditions.

## 3. Dehydration by means of total fasting

Referring to Table V, it is interesting to note the figures for "preformed water" and how they are related to the water balance the first 3 days of the fasting period. While the glycogen stores in the liver are being used up, the corresponding preformed water becomes available and this tends to retard the dehydration. Definite thirst did not occur until noon of the second day.

Another point of interest is the fact that on

the first day of recovery this subject gained 1257 grams in weight, in spite of the fact that he was still fasting and losing solids from his body. He was losing solids in two ways. He excreted 44.5 grams in the urine, and oxidized about 229 grams of protein and fat. We should expect, then, that the absolute gain in body water on that day ought to be close to the sum of these three figures, or 1530.5 grams. Table VI gives essentially the same value in column 8, namely 1538 grams. In other words, here is clear evidence, if any evidence is needed, that a thirsting individual can take up water and retain it without any concomitant intake of electrolytes or of any other solid matter. Wiley and Wiley (23) came to the conclusion that a dehydration of about 1.5 per cent of the body weight can occur without great disturbance of salt balance. Our data indicate that a change in hydration amounting to at least 2 per cent of the body weight can occur without a corresponding change in salt or solid content. In fact, if we correct for the fact that solutes were being lost while water was being gained, as in column 9 in Table VI, we arrive at 1929 grams for the 24-hour period in question, or 2.7 per cent of the body weight. This clearly indicates that for short periods of time water balance cannot always be estimated accurately from balances of Na and K.

As indicated in column 9 in Table VI, there was, during the 3 days of total fasting, a greater absolute reduction in the amount of body water than would be predicted purely on the basis of sodium and potassium balance. In other words, by the end of the dehydration period there was in the body enough fixed base (Na and K) to "hold" an additional 1919 grams of water. It is interesting to note that at the end of the following day, during which time water was allowed ad libitum and during which time Na and K were still being excreted, the body had regained and retained 1929 grams of water, almost precisely the theoretically predicted amount.

Once the subject was rehydrated, it was not possible to hyperhydrate him by forcing water. The body weight curve seemed to be unaffected by attempts at hyperhydration, although it had been markedly affected by dehydration and rehydration. Of course, the subject was not allowed

to drink for several hours immediately preceding each weighing, and was always weighed with an empty bladder.

#### 4. The significance of thirst in dehydration

As discussed, it is clear that thirst is not present in all types of dehydration. It is present in that type brought about by water deprivation. It may be completely absent in the extracellular, or salt-loss, type of dehydration. These facts fit in well with the concept that thirst depends upon cellular dehydration (24).

#### 5. Oliguria, anuria, and azotemia

Oliguria, anuria and azotemia have long been considered common manifestations in dehydrated patients, but the type of dehydration referred to has generally not been specified.

In the experiments described above it is noteworthy that in IIA and IIB, which illustrate the "thirst" type of dehydration, the daily volume of urine decreased to 510 cc. in one case and 430 cc. in the other. It will be noted in Experiment IIB (Table IV) that the urine volumes on the 3 days of water deprivation were 430, 450 and 445 cc., respectively, the lowest volume occurring on the first day. Obviously, in this case the degree of oliguria is not an accurate index of the extent of the dehydration. In two similar cases reported by Coller and Maddock (25) there was no accurate correlation between the degree of oliguria and the extent of dehydration. It appears that a certain minimum urinary volume is inevitable as long as blood pressure is high enough to insure glomerular filtration. On theoretical grounds we should rarely expect to find anuria from water deprivation alone.

In Experiments IA and IB the urine volumes were normal at all times because water was being adequately supplied. These experiments were terminated before the blood pressures dropped to dangerously low levels. It seems obvious that, if the salt loss had been allowed to continue until true shock occurred, the urine secretion might have ceased altogether for the simple reason that glomerular filtration cannot occur when the blood pressure is sufficiently low (26). It is a common observation that patients in profound shock usually have anuria.



It seems reasonable to conclude that in the "thirst" type of dehydration associated with simple water deprivation, oliguria should be expected, but not anuria; while in the "salt-loss" type, urinary excretion may vary from the normal to complete anuria.

Azotemia, when due to dehydration, is most simply explained as depending chiefly upon the degree and duration of the associated oliguria or anuria. The extent to which azotemia may depend upon other factors will not be discussed here.

#### SUMMARY AND CONCLUSIONS

1. Two distinct types of dehydration were produced in normal human subjects.

2. The results were quite comparable to those previously described in rabbits by Kerpel-Fronius and indicate that the two types differ from each other in mechanism of production, in manifestations, and in the treatment indicated.

3. Dehydration resulting from simple water deprivation is characterized by thirst and oliguria, does not lead to impairment of the circulation, and is completely relieved by the administration of water.

4. Dehydration resulting from abnormal salt loss results mainly in a loss of extracellular fluid, a reduction in plasma volume, and disturbances in the circulation. It is neither characterized by thirst nor relieved by the administration of salt-free fluids, but is promptly relieved by fluid containing sodium chloride.

5. A detailed study was made of the balances of energy, water, sodium, and potassium during a period of total fasting and recovery. The results indicate that during dehydration and recovery the balances of sodium and potassium do not necessarily correspond to the water balance over short periods of time.

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# CALCULATION OF THE VENOUS-ARTERIAL SHUNT IN CONGENITAL HEART DISEASE

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It is generally agreed that the chronic cyanosis of congenital heart disease is due essentially to a venous-arterial shunt which permits venous or un-aerated blood to mix with arterial or oxygenated blood. The amount of shunt is the chief factor which determines the degree of cyanosis and the severity of the condition in a given instance. Determination of the amount of the shunt in terms of percentage of the total blood flow is, therefore, of great prognostic importance. Unfortunately, the methods for such determinations hitherto employed require data difficult to obtain and involve tedious gas and blood analyses. The values which have been recorded have been viewed with skepticism by the investigators themselves, as noted by Abbott (1). Even were the results trustworthy, these methods are unsuitable for clinical use because of their complexity and because of the protracted and painful procedures they impose upon the patients. The method proposed in this communication is one of great simplicity; it involves but a single venipuncture, and requires but twenty minutes for its completion. The procedure determines whether or not a venous-arterial shunt is present, indicates the approximate magnitude of the shunt, and provides a method for determining the true pulmonary circulation time.

## METHOD

*First procedure.* A needle with a large bore (14 to 16 gauge) is inserted into the antecubital vein. One-half cubic centimeter of a 50 per cent solution of ether is injected, and the exact interval between the time of injection and the moment when the patient feels a prickly, burning sensation in the face is recorded. All injections are made as rapidly as possible at the beginning of inspiration. In the absence of a venous-arterial shunt this facial sensation is not experienced because all the ether is eliminated by the lungs. The occurrence of this sensation, on the other hand, testifies

to the entry of ether into the systemic circulation by way of the shunt (2). This ether circulation time thus measures the time that it takes for the blood to travel from the arm to the right side of the heart and thence by way of the shunt to the capillaries of the face.

*Second procedure.* Beginning with 0.1 cc. of a 50 per cent solution of saccharine, successively larger injections are given until the smallest amount which the patient can taste is determined. If 50 per cent or more of the circulating blood enters the shunt, the saccharine threshold time will be equal approximately to the "ether-shunt" time because both test substances have reached the head via the short circuit. In such a case, the prognosis is grave and a precise figure is not necessary, so the test is completed at this point.

If the saccharine threshold time is considerably longer than the "ether-shunt" time, it can be concluded that more than half the blood goes through the regular channel and the approximate percentage can be calculated in the following manner.

*Third procedure.* Successively larger amounts of saccharine are injected. The times will remain fixed at the level of the threshold time and will exceed the "ether-shunt" time by the interval required for the blood to traverse the lungs. When the injection is large enough to permit a detectable quantity of saccharine to reach the tongue by way of the shunt, the circulation time will suddenly become conspicuously shorter than in the previous estimations and will be found equal to the "ether-shunt" time.

In order to understand the method of arriving at the principle of calculation of the magnitude of the shunt, it is necessary to add a few explanatory remarks. When the smallest amount of saccharine that can be tasted is determined for a patient with a venous-arterial shunt of less than 50 per cent, a certain amount of the saccharine enters the shunt and reaches the tongue, but the concentration is not sufficient to cause the material to be tasted. The true value for the threshold of taste in this instance, therefore, is not the total amount of saccharine injected, but that amount less the quantity which reaches the tongue by the short route, *i.e.*, by way of the shunt. Except in the presence of complications, such as heart failure or polycythemia, the circulation time thus

<sup>1</sup> Endowed by grants from the Beaumont Trust Fund and Henry Dazian Foundation for Medical Research.



# CALCULATION OF THE VENOUS-ARTERIAL SHUNT IN CONGENITAL HEART DISEASE

By MYRON PRINZMETAL

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(Received for publication July 7, 1941)

It is generally agreed that the chronic cyanosis of congenital heart disease is due essentially to a venous-arterial shunt which permits venous or un-aerated blood to mix with arterial or oxygenated blood. The amount of shunt is the chief factor which determines the degree of cyanosis and the severity of the condition in a given instance. Determination of the amount of the shunt in terms of percentage of the total blood flow is, therefore, of great prognostic importance. Unfortunately, the methods for such determinations hitherto employed require data difficult to obtain and involve tedious gas and blood analyses. The values which have been recorded have been viewed with skepticism by the investigators themselves, as noted by Abbott (1). Even were the results trustworthy, these methods are unsuitable for clinical use because of their complexity and because of the protracted and painful procedures they impose upon the patients. The method proposed in this communication is one of great simplicity; it involves but a single venipuncture, and requires but twenty minutes for its completion. The procedure determines whether or not a venous-arterial shunt is present, indicates the approximate magnitude of the shunt, and provides a method for determining the true pulmonary circulation time.

## METHOD

*First procedure.* A needle with a large bore (14 to 16 gauge) is inserted into the antecubital vein. One-half cubic centimeter of a 50 per cent solution of ether is injected, and the exact interval between the time of injection and the moment when the patient feels a prickly, burning sensation in the face is recorded. All injections are made as rapidly as possible at the beginning of inspiration. In the absence of a venous-arterial shunt this facial sensation is not experienced because all the ether is eliminated by the lungs. The occurrence of this sensation, on the other hand, testifies

to the entry of ether into the systemic circulation by way of the shunt (2). This ether circulation time thus measures the time that it takes for the blood to travel from the arm to the right side of the heart and thence by way of the shunt to the capillaries of the face.

*Second procedure.* Beginning with 0.1 cc. of a 50 per cent solution of saccharine, successively larger injections are given until the smallest amount which the patient can taste is determined. If 50 per cent or more of the circulating blood enters the shunt, the saccharine threshold time will be equal approximately to the "ether-shunt" time because both test substances have reached the head via the short circuit. In such a case, the prognosis is grave and a precise figure is not necessary, so the test is completed at this point.

If the saccharine threshold time is considerably longer than the "ether-shunt" time, it can be concluded that more than half the blood goes through the regular channel and the approximate percentage can be calculated in the following manner.

*Third procedure.* Successively larger amounts of saccharine are injected. The times will remain fixed at the level of the threshold time and will exceed the "ether-shunt" time by the interval required for the blood to traverse the lungs. When the injection is large enough to permit a detectable quantity of saccharine to reach the tongue by way of the shunt, the circulation time will suddenly become conspicuously shorter than in the previous estimations and will be found equal to the "ether-shunt" time.

In order to understand the method of arriving at the principle of calculation of the magnitude of the shunt, it is necessary to add a few explanatory remarks. When the smallest amount of saccharine that can be tasted is determined for a patient with a venous-arterial shunt of less than 50 per cent, a certain amount of the saccharine enters the shunt and reaches the tongue, but the concentration is not sufficient to cause the material to be tasted. The true value for the threshold of taste in this instance, therefore, is not the total amount of saccharine injected, but that amount less the quantity which reaches the tongue by the short route, *i.e.*, by way of the shunt. Except in the presence of complications, such as heart failure or polycythemia, the circulation time thus

<sup>1</sup> Endowed by grants from the Beaumont Trust Fund and Henry Dazian Foundation for Medical Research.

determined should be within normal limits, inasmuch as that part of the saccharine which is ultimately tasted reaches the tongue in the usual manner, *i.e.*, after traversing the pulmonary circulation.

As previously stated, the saccharine circulation time is found to remain constant when increasing amounts are injected, until a point is reached when the circulation time is suddenly found to be conspicuously shorter. When this occurs, it signifies that a sufficient amount of saccharine has reached the tongue without traversing the pulmonary circulation and that the true threshold value for saccharine is now represented by the amount of this substance which reaches the tongue directly by way of the shunt.

With these facts in mind, and the necessary determinations having been made, the amount of shunt in terms of percentage of total blood flow can be calculated with the aid of the following simple calculation:

If  $A$  is the smallest amount of saccharine which can be tasted in the longer circulation time period, and  $C$  is the smallest amount of saccharine that can be tasted in the shorter time period; then  $\frac{A}{A+C}$  would equal the amount of shunt in terms of percentage of the total blood flow, which may be called  $k$ . It is not necessary to determine the actual threshold value of saccharine taste sensation but only to note that it would be equal to either  $A - kA$  or to  $kC$ , which therefore equal each other. If  $A - kA = kC$ , then  $A = kC + kA$  or  $A = k(C + A)$  or  $k = \frac{A}{A+C}$ .

This method also enables the observer to determine the true pulmonary circulation time, *i.e.*, the time required for the blood to travel from the right side of the heart to the left side of the heart by way of the pulmonary circulation. Inasmuch as the longer (saccharine threshold) circulation time measures the time that it takes for the blood to travel from the arm to the tongue *after traversing the lungs*, and the shorter saccharine circulation time measures the time that it takes for the blood to travel from the arm to the tongue *without traversing the lungs*, the difference between these two circulation times measures the time that it takes for the blood to traverse the

pulmonary circulation. In the past it has been possible to determine the true pulmonary circulation time only by the complex radon seed method of Blumgart and Weiss (6). The latter, however, cannot be used in the presence of a venous-arterial shunt. On the other hand, the method described in this paper is not suitable for cases without venous-arterial shunt.

## RESULTS

The degree of shunt in cyanotic patients with congenital heart disease was determined in four instances (Table I). The shunt was less than 50 per cent in each instance, necessitating the use of the third procedure. The percentage of shunt in each case was 11.8, 21, 13, and 21, respectively. The true pulmonary circulation times were 18, 10, 18, and 14 seconds, respectively. Since the total circulation times averaged 27.5 seconds, the true pulmonary circulation time was approximately 65 per cent of the total circulation time.

One patient (Case II) did not taste saccharine when administered intravenously and a 50 per cent solution of calcium levulinate was used, the cutaneous burning sensation being utilized as the end point. One subject had Hodgkin's disease and died of this illness six weeks after the test was performed. At autopsy, in addition to the Hodgkin's disease, a patent intraventricular septal defect was found, the right ventricle was hypertrophied, the pulmonary artery was dilated to twice the size of the aorta, and marked pulmonary arteriosclerosis was present. Apparently, the greatly increased pressure in the right ventricle resulting from the pulmonary arteriosclerosis caused a venous-arterial shunt instead of the usual arterial-venous shunt through the septal defect. It was considered hazardous to make antemortem diagnoses in the other cases, but such precise information is not necessary for this study.

An example of the method follows (Case I):

### First procedure

0.3 cc. ether—prickly sensation of face, seventeen seconds.

This demonstrates that a venous-arterial shunt is present. Seventeen seconds is the time required for the ether to travel from the arm through the shunt, avoiding the lungs, to the blood vessels of the face.

TABLE I

Case	Age	Sex	Cy- anosis	Red blood count	Hemo- globin	Ether shunt time	Longer circulation time (includes pulmonary circulation)	Smallest amount of 50 per cent saccharine for longer circulation time period	Shorter circulation time as determined by 50 per cent saccharine through shunt including pulmonary circulation	Smallest amount of saccharine for shorter circulation time	True pul- mo- nary cir- cu- lation time	Per- cent- age of shunt
					per cent	seconds	seconds	cc.	seconds	cc.	seconds	per cent
I	27	M	++	8,720,000	116	17	32	0.4	14	3	18	11.8
II	17	M	++	5,640,000	97	16	15	1 50 per cent solution of calcium levulinate	5	3.75 50 per cent solution of calcium levulinate	10	21
III	20	M	++			11	32	1.0	14	7	18	13
IV	23	F	++	5,780,000	75	14	31	0.8	17	3	14	21

*Second procedure*

- 0.1 cc. 50 per cent saccharine....not tasted.  
 0.2 cc. 50 per cent saccharine....not tasted.  
 0.3 cc. 50 per cent saccharine....not tasted.  
 0.4 cc. 50 per cent saccharine....sweet taste, 30 seconds.  
 0.4 cc. 50 per cent saccharine....sweet taste, 34 seconds.  
 0.3 cc. 50 per cent saccharine....not tasted.  
 0.4 cc. 50 per cent saccharine....sweet taste, 35 seconds.

The patient could not taste 0.3 cc. of a 50 per cent solution of saccharine, but always tasted 0.4 cc.; 0.4 cc. is therefore the threshold volume. Since the saccharine threshold time (thirty to thirty-five seconds) is so much longer than the "ether-shunt" time (seventeen seconds), it can immediately be concluded that most of the blood goes through the lungs, by way of the normal circulation, and less than one-half of the cardiac output goes through the shunt. The approximate per cent can now be determined by injecting increasingly larger amounts of saccharine until the shorter circulation time is attained.

*Third procedure*

- 2.0 cc. saccharine.....sweet taste, 32 seconds.  
 2.5 cc. saccharine.....sweet taste, 31 seconds.  
 2.75 cc. saccharine.....sweet taste, 32 seconds.  
 3.00 cc. saccharine.....sweet taste, 14 seconds.

From the determinations, it is apparent that in 2.75 cc. of 50 per cent saccharine, the volume going through the shunt was not sufficient to cause the taste sensation since the circulation time was 32 seconds. In 3.0 cc., however, enough of the solution went through the shunt to cause the sweet taste to occur at a time approximately equal to the "ether-shunt" time, and much faster than the previous injections. Since the taste threshold of saccharine is 0.4 cc., the approximate volume of the shunt can now be determined by means of the formula:

$$K = \frac{A}{A + C}$$

Volume of shunt:

$$\frac{0.4}{0.4 + 3.0} = 11.8 \text{ per cent.}$$

The true pulmonary circulation time can be determined by subtracting the shorter circulation time (fourteen sec-

onds) from the total circulation time (thirty-two seconds), which excludes the pulmonary circulation. In this case, it is approximately eighteen seconds.

## DISCUSSION

The shunt in all four patients is quite small and this undoubtedly explains the comparative well-being of these subjects in spite of the malformation of their hearts. The method is probably not suitable for children unless they are able to cooperate. In the very few cases in which the shunt was determined previous to this study, larger figures were obtained (3, 4, 5).

The prolongation of the circulation time in some of our patients may be due to the polycythemia. The effect of this factor on the circulation time has been previously noted (7). Persistent cyanosis in those who have congenital heart disease is a result of (1) a septal defect in the presence of pulmonary stenosis, (2) a complete absence of the cardiac septa, or (3) the transposition of the great trunks. In addition, there are other rarer conditions which are occasionally responsible. The method described in this communication for determining the magnitude of the venous-arterial shunt does not indicate which of the above conditions exists in a given case. In the absence of characteristic findings in the cases herein reported, it was considered hazardous to make a precise anatomical diagnosis.

This method cannot give exact figures if the shunt is greater than 50 per cent, but the knowledge that a shunt of this degree exists is of sufficient value to make a precise figure unnecessary. The streaming of the saccharine through the lungs should be greater than that going directly through



the shunt. The solution going through the lungs becomes more diluted than that going through the shunt. This introduces an error, the magnitude of which is unknown. This factor affects only the third, not the first two procedures. It is therefore apparent that, if the shunt is less than 50 per cent, the accuracy of the figure obtained can be considered only approximate, but this approximate value should be extremely useful from a prognostic point of view.

#### CONCLUSIONS

A simple method has been described for determining the presence of a venous-arterial shunt in congenital heart disease, the magnitude of the shunt, and the true pulmonary circulation time in the presence of shunt.

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# THE PLASMA LEVELS OF VITAMIN A AFTER THE INGESTION OF STANDARD DOSES: STUDIES IN NORMAL SUBJECTS AND PATIENTS WITH CIRRHOSIS OF THE LIVER<sup>1</sup>

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In a previous study it was found that the livers of patients with cirrhosis of the liver contained much less vitamin A than did the livers of normal subjects (1). The concentration of carotene was also lower in the livers of the patients with cirrhosis but was not as strikingly reduced as was the liver vitamin A. More recently, we have observed that the plasma levels of vitamin A and carotene were lower in patients with cirrhosis of the liver than in normal subjects (2). Apparently, the low level of vitamin A in the plasma reflected the diminished amounts of vitamin A in the livers of patients with cirrhosis. This suggested that there might be an appreciable difference in the plasma levels of vitamin A following the administration of a standard dose of vitamin A to normal subjects and to patients with cirrhosis.

Observations were therefore made on a group of normal subjects, on 5 patients with cirrhosis, and on a patient with acute catarrhal jaundice. The patients with cirrhosis gave histories of chronic alcoholism.

## PROCEDURE

The normal group consisted of 12 male adults. Vitamin A was given by mouth in the form of a cod liver oil concentrate. Four of the normal subjects received the equivalent of 20,000 U.S.P. units of vitamin A. This was 1 cc. of the cod liver oil concentrate. Eight of the normal subjects were given an amount of the concentrate equivalent to 100,000 U.S.P. units of vitamin A and this dose was also given to the 5 patients with cirrhosis and the patient with acute catarrhal jaundice. Prior to the administration of the vitamin, the range of the plasma levels of vitamin A was established in each subject by repeated daily determinations. Following the oral administration of cod liver oil, the plasma levels of vitamin A and carotene were determined at intervals of 3, 6, 9, 12, and 24 hours. When it was found that, in the normal subjects, no further increase in the level of vitamin A

occurred at 9 and 12 hours, the determinations at these intervals were omitted in the normals.

Vitamin A, after extraction from the plasma, was determined in the photoelectric colorimeter, using antimony trichloride. The method, with slight modifications, was similar to that reported by Kimble (3). The carotene concentration was determined in the photoelectric colorimeter by the method described by Stueck *et al.* (4). In order to calculate the concentration of vitamin A in the plasma in U.S.P. units, calibration curves were done, using a standard U.S.P. reference oil that contained 1700 U.S.P. units of vitamin A per gram. Calibration curves were also done for beta-carotene with antimony trichloride. To correct for the blue color due to carotene in the extracted plasma, the concentration of carotene in the petroleum ether extract of the plasma was determined and subtracted from the galvanometer reading for the total blue value obtained. The result gave the value for vitamin A.

## RESULTS

The effects of the cod liver oil concentrate on the plasma levels of vitamin A and carotene are summarized in Table I. Figure 1 shows the individual plasma levels of vitamin A in the normal subjects receiving 100,000 U.S.P. units of vitamin A and in the 5 patients with cirrhosis of the liver.

Following the administration of 20,000 U.S.P. units of vitamin A to the 4 normal subjects, there was a rise in the plasma level of vitamin A, which occurred in 3 cases after 6 hours and in 1 case after 3 hours. The average increase in the plasma level was 84 U.S.P. units. After 12 hours the plasma levels in all 4 cases had returned to about the original fasting levels. During this time there were no changes in the plasma levels of carotene. In fact, the carotene remained quite constant.

The 8 other normal subjects each received 100,000 U.S.P. units of vitamin A. Following this dose there was a much sharper increase in the plasma levels of vitamin A, as is shown in the figure. The peak of this increase was reached

<sup>1</sup> This research was aided by a grant from The Milbank Memorial Foundation.

TABLE I

*Effect of large doses of vitamin A in the form of a cod liver oil concentrate on the plasma levels of vitamin A in normal subjects, in patients with cirrhosis of the liver and in a case of acute catarrhal jaundice*

Group	Number of cases	Vitamin A dosage	Plasma vitamin A (USP units per 100 cc.)						Plasma carotene (mgm. per cent)					
			Hours after cod liver oil taken						Hours after cod liver oil taken					
			0	3	6	9	12	24	0	3	6	9	12	24
Normals.....	4	USP units 20,000	140	190	214	165	154	151	0.148	0.148	0.148	0.148	0.148	0.150
Normals.....	8	100,000	168	423	374	243*	210*	172	0.110	0.111	0.109			0.108
Cirrhosis.....	5	100,000	44	141	111	84	74	47	0.079	0.073	0.076	0.075	0.075	0.077
Acute catarrhal jaundice.....	1	100,000	63	95	118	105	105		0.040	0.040	0.035	0.035	0.035	0.035

\* Average of 2 cases. In subsequent curves the 9- and 12-hour determinations were omitted, since it was clear that no further rise of the plasma level occurred after 6 hours.

after 3 hours in 5 of the cases and after 6 hours in 3 cases. At the end of 24 hours the plasma levels were at or only slightly above the original fasting values. The average total increase in the plasma level of vitamin A after the ingestion of 100,000 U.S.P. units was almost four times as great as the average increase after the ingestion of 20,000 U.S.P. units of the vitamin. The administration of this large dose of vitamin A in the form of a cod liver oil concentrate had no effect on the plasma levels of carotene in any of the subjects.

In the 5 cases of cirrhosis of the liver and in the 1 case of acute catarrhal jaundice the larger dose of vitamin A was given (100,000 U.S.P. units). None of the patients with cirrhosis was jaundiced, but 4 of them had ascites. In 3 of the 5 cases, the fasting levels of vitamin A in the plasma were very low, *i.e.*, 18 to 48 U.S.P. units per 100 cc. of plasma, and in the other 2 cases the fasting plasma levels were below the normal range of 88 to 188 U.S.P. units per 100 cc. of plasma, which we have previously observed (2). The ingestion of 100,000 U.S.P. units of vitamin A was followed by a rise in the plasma levels of vitamin A in the patients with cirrhosis, but the increase was *much less* than that which occurred in the normal subjects following the same dose. The peak of the increase occurred in most of the cases at the third hour and averaged 99 U.S.P. units, whereas in the normal subjects the average increase was 318 U.S.P. units per 100 cc. of plasma. In the patients with cirrhosis, as in the normals, the plasma levels of vitamin A re-

turned to about the original values after 24 hours. There was no change in the plasma levels of carotene during the time of the observations. In all the patients with cirrhosis the plasma levels of carotene were lower than in the normal subjects.

In the one case of jaundice unassociated with cirrhosis of the liver, the response to the ingestion of 100,000 U.S.P. units of vitamin A was similar to that observed in the patients with cirrhosis (Table I). The total rise in the plasma level of vitamin A was 55 U.S.P. units per 100 cc. of plasma. In this patient the level of carotene was very low, 0.040 mgm. per cent, and the administration of the cod liver oil concentrate had no effect on the level of carotene.

The amount of the cod liver oil concentrate used was only 5 cc., so that it seemed unlikely that the diminished response in the plasma level of vitamin A in the patients with cirrhosis of the liver was due to any impairment in absorption. However, to rule this question out and to satisfy ourselves that patients with cirrhosis of the liver could be saturated with vitamin A, one patient with cirrhosis was fed 300,000 U.S.P. units of vitamin A daily. The plasma level before vitamin A was begun was 40 U.S.P. units per 100 cc. After 3 days the plasma level rose to 118 U.S.P. units per 100 cc. Vitamin A was continued in doses of 100,000 U.S.P. units daily for 9 weeks and then, following the test dose, the plasma levels of vitamin were determined at the usual intervals. The level of vitamin A in the plasma rose 220 U.S.P. units. This response, although not quite as great as the average observed in the

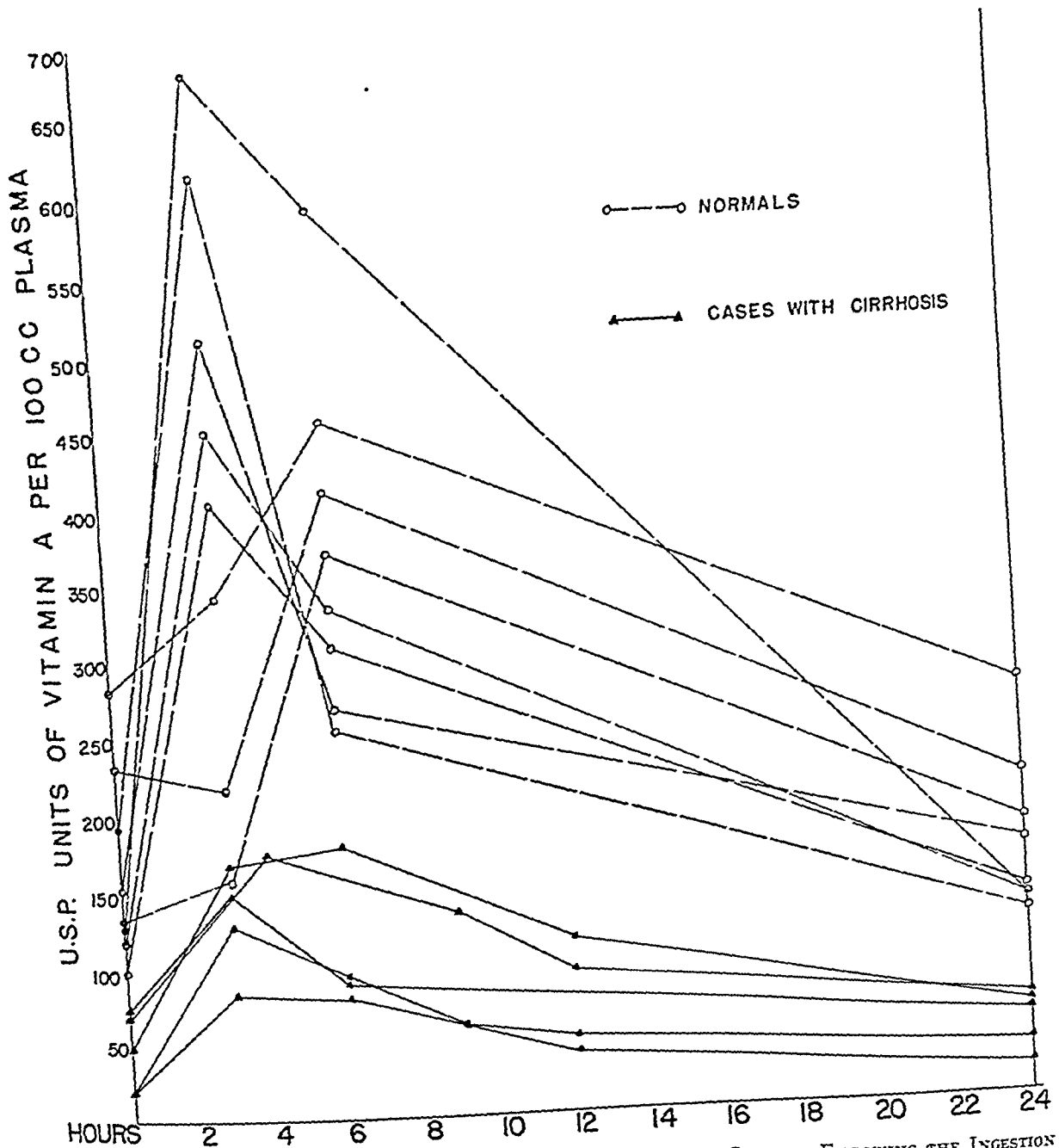


FIG. 1. THE PLASMA LEVELS OF VITAMIN A IN NORMAL AND CIRRHOTIC SUBJECTS FOLLOWING THE INGESTION OF 100,000 U.S.P. UNITS OF VITAMIN A

normals, was more than twice as great as that observed in the 5 patients with cirrhosis and, as can be seen in the figure, was similar to the rise obtained in some of the normal subjects.

#### DISCUSSION

Following the administration of large doses of vitamin A in the form of a cod liver oil concen-

trate to human subjects, the plasma levels of vitamin A rose in every instance. In the normal group, the extent of this rise was dependent upon the amount of vitamin A fed and, when five times the amount of vitamin A was ingested, the average increase was approximately four times as great. In all cases the plasma values returned to the original fasting levels after 24 hours.

The administration, however, of 100,000 U.S.P. units of vitamin A to patients with cirrhosis of the liver resulted in a much smaller rise in the plasma levels of the vitamin than in the normal subjects. This result was not unexpected in view of the previous observation that the concentration of vitamin A in the liver was decreased in patients with cirrhosis of the liver (1). Undoubtedly, a greater portion of the ingested vitamin was retained by the liver in the patients with cirrhosis than in the normal subjects. It seemed probable that certain other pathological conditions of the liver might also influence the liver stores of vitamin A. This was suggested by the fact that, in the patient with acute catarrhal jaundice, the plasma level of vitamin A was low and the response to the test dose was similar to that observed in the patients with cirrhosis.

In neither the normal subjects nor in the patients with cirrhosis of the liver were the levels of carotene in the plasma changed following the administration of the cod liver oil concentrate. There was no carotene in the concentrate and, apparently, the level of carotene in the plasma will only be altered when carotene itself is administered.

Pett and LePage have reported (5) a definite correlation between the vitamin A level in the blood and the visual test for vitamin A deficiency. Haig *et al.* (6) noted that, in patients with cirrhosis of the liver, there was an impairment in dark adaptation. Our findings, therefore, of a low plasma level of vitamin A in patients with cirrhosis of the liver would fit in with the observations of Haig, Hecht and Patek and would corroborate the observations of Pett and LePage that there is a correlation between the vitamin A level in the blood and the visual recovery time. In later work Patek and Haig (7) reported that dark adaptation was improved in patients with cirrhosis of the liver following the feeding of vitamin A in the form of cod liver oil. This also fits in with the results that we obtained in the one patient with cirrhosis of the liver who, following the daily ingestion of cod liver oil, had a normal plasma level of vitamin A and a normal response to a standard dose of the vitamin.

The results obtained in these studies suggest

that there is a deficiency of vitamin A in patients with cirrhosis of the liver which is reflected in the low plasma level of vitamin A. This deficiency may be partly due to the liver damage and it may also be due to the fact that vitamin A is normally ingested in the form of its precursor carotene which, in the presence of liver damage, may not be converted to vitamin A.

The administration of standard doses of vitamin A to normal subjects and to patients with cirrhosis of the liver serves as a "tolerance test." This procedure might well be used as a measure of vitamin A deficiency in patients with cirrhosis of the liver or in patients with liver damage.

#### SUMMARY

Standard doses of cod liver oil in the form of a cod liver oil concentrate were given to 12 normal subjects, to 5 patients with cirrhosis of the liver and to 1 case with acute catarrhal jaundice. Four of the normal subjects received a dose of 20,000 U.S.P. units of vitamin A and 8 received a dose five times as great (100,000 U.S.P. units of vitamin A). The larger dose resulted in a greater increase in the plasma levels of vitamin A determined at intervals over a 24-hour period.

The patients with cirrhosis of the liver and the patient with jaundice were given 100,000 U.S.P. units of vitamin A. The rise in the plasma level of vitamin A, compared to the rise which occurred in the plasma levels in the normal subjects, was much less in the patients with liver damage.

The results suggest that a test of this type may serve as an index of the degree of vitamin A deficiency in patients with cirrhosis of the liver or with other diseases of the liver.

The findings of a low plasma level of vitamin A in patients with cirrhosis of the liver fit in with the previous observations of a lowered concentration of vitamin A in the livers of such patients.

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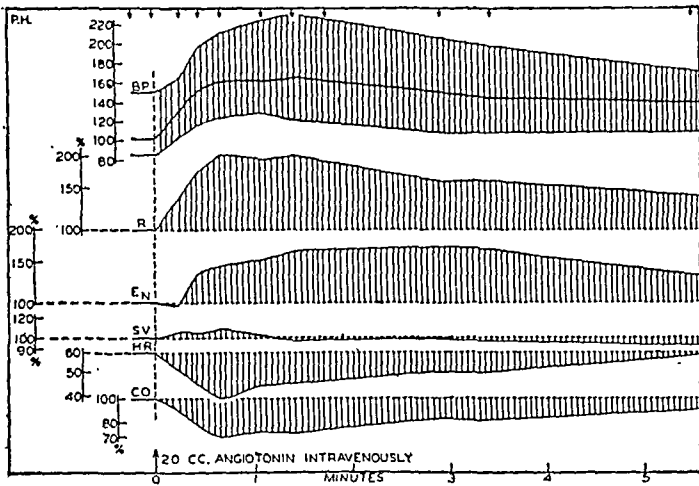


FIG. 1. HEMODYNAMIC EFFECTS OF A SINGLE INTRAVENOUS INJECTION OF ANGIOTONIN

$P_s - P_d/DP_{md}$  (where  $P_s$  is the systolic pressure,  $P_d$  the diastolic pressure,  $D$  is duration of arterial diastole or of the fall of pressure in the arterial pulse, and  $P_{md}$  is the mean pressure during  $D$ ), and since  $\gamma_n R = E_n$ , as shown below<sup>2</sup>,  $E_n = R(P_s - P_d/DP_{md})$ .  $E_n$  is expressed as dynes/cm.<sup>2</sup>

We wish to acknowledge our debt to Dr. Seth Hirsh and Mr. Myron Schwartzchild of the Department of Roentgenology of this School for aid and direction in the study of the effect of angiotonin on heart size and pulsation. Kymoroentgenograms and cardiocairograms were obtained before administration of the drug and again at the height of the pressor effect. The cardiocairogram was timed to picture the heart shadow in mid-diastole.

### RESULTS

Figure 1 illustrates the changes which occurred to a greater or lesser degree in all experiments. Peripheral resistance and mean arterial pressure rose consistently, with an increase in pulse pressure. In the experiment illustrated, stroke vol-

ume decreased—though this was not invariably the case, some subjects showing virtually no change—and in no instance did stroke volume increase.

tion of natural gamma,  $\gamma_n$ , which is practically most useful. In the determination of  $\gamma_n$ , the diastolic stroke flow ( $i$ ) is zero:

$$\int_S^T i(t)dt = \int_S^T \left( \frac{P'}{E} + \frac{P}{R} \right) dt = \int_S^T (P' + \gamma_n P) dt = 0,$$

where  $S$  is time of systole and  $T$  is the end of the pulse period.

$$\therefore \gamma_n = - \frac{\int_S^T (P') dt}{\int_S^T P dt} = \frac{P_s - P_d}{\int_S^T P dt},$$

where  $P_s$  is systolic pressure,  $P_d$  is diastolic pressure,  $\int_S^T P dt$  is the product of mean pressure during fall of pressure from systolic peak to end of diastole and duration of this fall of pressure.

Thus it can be seen that  $E_n = \gamma_n R$ .

Because of uncertainties in the absolute values of the stroke volume, as determined by the ballistocardiograph, this measurement and all dependent measurements ( $CO$ ,  $E_n$ , and  $R$ ) are expressed as percentile changes relative to the respective control values.

In four single injection experiments, cardiac output was substantially reduced, the reduction being the result of decreased heart rate, which was invariable, and such reduction in stroke volume as may have been present. With a single exception (which is shown in Figure 3),  $E_n$  increased markedly, indicating decreased distensibility of the central arterial reservoir.

Figure 2 illustrates the effect of the continuous infusion of angiotonin (three experiments). The hemodynamic changes were similar to those induced by a single injection, except that bradycardia is not evident and the decrease in cardiac output is rather less.

X-ray studies of the heart shadow in four additional subjects following intravenous injection of angiotonin revealed no consistent changes, two subjects showing a slight increase in all diameters, one showing no change, and one showing a slight

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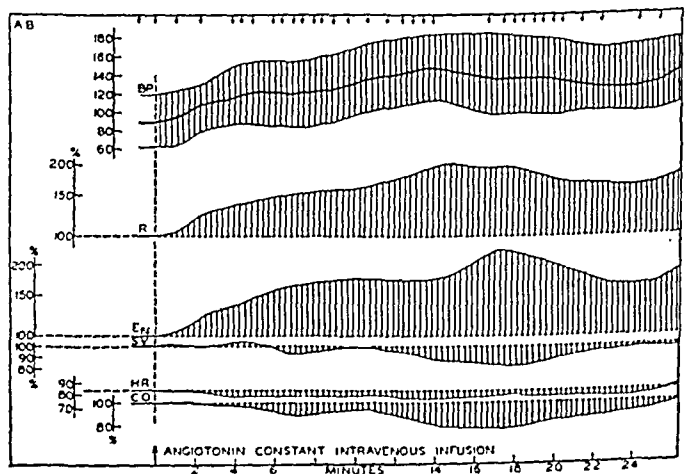


FIG. 2. HEMODYNAMIC EFFECTS OF THE CONTINUOUS INTRAVENOUS INFUSION OF ANGIOTONIN

Symbols same as in Figure 1.

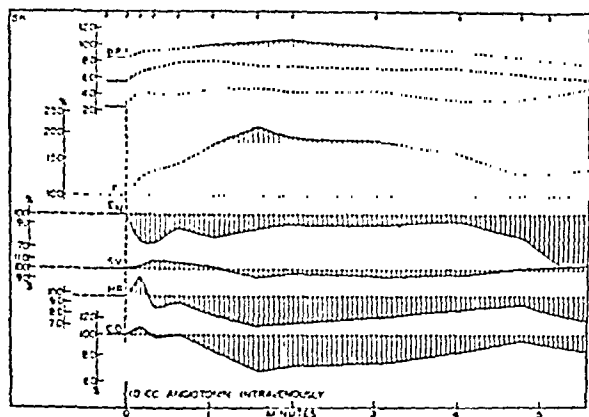


FIG. 3. AN ANOMALOUS INSTANCE WHERE  $E_n$  WAS APPARENTLY DECREASED AFTER ANGIOTONIN  
A possible explanation is discussed in the text.

decrease in all diameters. However, kymorontgenograms showed a consistent decrease in amplitude of ventricular waves, which was marked in three cases, amounting to as much as 50 per cent. This apparently indicates a decreased emptying of the heart and, since the diastolic volume was not appreciably increased, a decrease in stroke volume. This qualitatively confirms our observations with the ballistocardiograph. There was little change in amplitude of auricular waves.

An interesting change in pulse pressure contour, consisting of the development of a secondary pre-dicrotic wavelet which was apparently a reflected wave, made its appearance approximately ten seconds after injection of the drug. This change was often associated with sudden, marked slowing and irregularity of the pulse.

No change in the electrocardiogram was observed.

#### DISCUSSION

Our data show that in man angiotonin is a definitely pressor substance, producing an elevation in systolic, diastolic and mean pressure, with a widening of pulse pressure. The increase in blood pressure appears to arise out of the increased peripheral resistance, presumably occasioned by arteriolar constriction in as yet undetermined sites.

The efficient elasticity modulus ( $E_n$ ) is found to increase in every case save one (Figure 3), which will be discussed below. This indicates that the distensibility of the central arterial reser-

voir decreases following the administration of angiotonin. It is, however, uncertain what factors operate to bring about this change. It may be that angiotonin acts upon the smooth musculature of the central arteries in the same manner that it acts upon the arterioles, increasing the rigidity of these arteries and thereby decreasing their distensibility. It is, however, well known that the distensibility of arteries decreases with increasing distention, and it may be expected that, when the resistance to outflow from the central arteries is increased by vasoconstriction, the cardiac output remaining relatively constant, increased distention of these arteries will occur and will be reflected in an increase in  $E_n$ . It is difficult at the present time to differentiate between these alternative explanations. It may be noted that if angiotonin acts directly on the central arteries to decrease their distensibility (in the sense of active contraction), we would expect their distensibility to be reduced more than would be the case if increased filling were the only factor. Comparison of our data with the curves published by Bramwell, McDowall and McSwiney (9) for muscular arteries reveals that the decrease in distensibility in our data is less than is to be expected from the equivalent changes in diastolic pressure. This fact might be interpreted as indicating that the distensibility-volume relations are different in the central arterial reservoir and the muscular arteries studied by Bramwell, McDowall and McSwiney, in which view any direct action of angiotonin on these arteries must be slight. We cannot, moreover, rule out a reflex mechanism, such as that observed in the dog by Dow and Hamilton (10), which on elevation of blood pressure increases the distensibility of the aorta. In any case the increase in pulse pressure observed in all subjects, and illustrated in Figures 1 and 2, is entirely the result of the change in distensibility of the central arterial reservoir, whatever its origin, and not the result of an increase in stroke volume.

We have mentioned that on one occasion (Figure 3)  $E_n$  fell following administration of one cc. of angiotonin intravenously. Two cc. in the same individual caused an elevation of  $E_n$ . It does not seem likely that distensibility of the central arterial reservoir increased in the face of disten-



tion in this reservoir, as revealed by a slight elevation of diastolic pressure; it is more likely that this case emphasizes the desirability of caution in interpreting mathematical analysis of the physiological forces which are involved. The calculated values involve several assumptions and yield what at best are only approximations which are useful for showing directional changes and indicating the magnitude of these changes. For example, the determination of  $\gamma_n$  is apparently valid only in pulse pressure curves which show a slight-to-moderate degree of dicrotism, since the occurrence of a marked dicrotic notch causes an apparent decrease in  $P_{md}$ , thus elevating  $E_n$ . With the exception of the subject illustrated in Figure 3, the pulse pressure curves analyzed here have shown only a slight dicrotism, but in that subject dicrotism occurred to a marked degree during the control period, associated with marked hypotension. Consequently, the control values of  $E_n$  are relatively high, and fall abruptly to a lower level after angiotonin, when dicrotism is reduced. Thus the change in pulse form (degree of dicrotism) may explain the apparent drop in  $E_n$  following the administration of angiotonin. Had excessive dicrotism not been present in the control periods, the control value of  $E_n$  might have been much lower and the changes following angiotonin might have resembled those observed in the other subjects.

The validity of  $E_n$  as an index of elasticity changes is supported by the fact that conversion of this value to pulse wave velocity, using Bramwell and Hill's (11) equation where pulse wave velocity =  $3.57/\sqrt{\text{per centile distensibility}}$ , gives velocities similar to those found in the literature (12, 13) for the central arteries. In addition, Apéria (8) has shown that  $E_n$  increases with age, an expected physiological result not obtainable by other indirect measurements of elasticity. Further studies of the relation between pulse wave velocity and  $E_n$  are in progress.

Bradycardia has in nearly every case been the chief cause for the reduction in cardiac output; presumably, the bradycardia represents vagal slowing, elicited through the carotid sinus and aortic depressor reflexes. Where the pressure rises slowly (Figure 2) no bradycardia ensues. In no instance did stroke volume increase, a rather surprising result since Wilkins (personal communi-

cation) has found a marked increase in cubital venous pressure, and Cournand and Ranges (unpublished data) have found a marked rise in right auricular pressure, after angiotonin administration. Since x-ray studies fail to show cardiac dilatation, it is inferred that the failure of the heart to increase its stroke volume in the face of increased venous pressure is attributable to increased "cardiac tone." This conclusion is further supported by the demonstration of decreased diastolic size of the isolated mammalian heart in response to angiotonin (3).<sup>3</sup>

#### SUMMARY

1. Cardiac output, mean arterial pressure, peripheral resistance and efficient elasticity modulus have been determined following intravenous administration of angiotonin.

2. In all experiments, mean arterial pressure and peripheral resistance rose sharply. The pulse pressure tended to widen. With one exception, the efficient elasticity modulus rose sharply. Cardiac output fell as a result of a marked bradycardia. There was little change in stroke volume and, where a change occurred, it was in the direction of a decrease. Response to small single injections and to continuous intravenous infusions differed only in the absence of bradycardia in the latter.

3. Kymoroentgenogram and cardiocairogram studies revealed little change in heart size. However, there was present a consistent decrease in amplitude of ventricular waves.

4. It is concluded that angiotonin acts directly upon the musculature of the cardiovascular system, producing arteriolar vasoconstriction and possibly increased "cardiac tone." Whether the distensibility of the central arterial reservoir is specifically decreased by angiotonin, or whether the observed change is attributable simply to increased distention of the central arteries, cannot be answered with certainty from the present data.

We wish to acknowledge our indebtedness to Miss Betty J. Crawford for her aid in computations.

<sup>3</sup> Wilkins and Duncan (J. Clin. Invest., 1941, 20, 442) have shown that angiotonin decreases the vital capacity. The above discussion should perhaps be qualified in the light of this fact. Concerning cardiac tone see Bibliography (14).

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# THE NATURE OF THE ARTERIAL HYPERTENSION PRODUCED IN NORMAL SUBJECTS BY THE ADMINISTRATION OF ANGIOTONIN<sup>1</sup>

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It is now established that sustained arterial hypertension can be produced experimentally in animals by interference with the blood supply of the kidney (1, 2, 3). Furthermore, this experimental hypertension can be relieved either by removing the interference or by excising the entire offended kidney (3, 4). There seems little doubt that this type of hypertension has a clinical counterpart in the naturally occurring hypertension of certain patients whose arterial pressure has been lowered after removal of an "ischemic" kidney (5 to 9).

It has also been demonstrated that the experimental hypertension associated with renal "ischemia" is humoral in nature (4, 10, 11), and probably due to the release of abnormal amounts of pressor substances by the offended kidney (11, 12, 13). This revived interest in the original work of Tigerstedt and Bergman (14) who in 1898 prepared from kidneys a pressor extract which they called "Renin." Modern methods of purification and analysis have revealed that the pressor agent in Renin is probably an enzyme (15, 16) which reacts with a pseudoglobulin fraction of blood to form a vasoconstrictor substance called "Angiotonin" (17) or "Hypertensin" (18).

Evidence is accumulating that a vasoconstrictor substance similar to, if not identical with, "Angiotonin" or "Hypertensin" is present in abnormal amounts in the blood of animals with experimentally produced renal "ischemia", and of certain patients with naturally occurring hypertension (18, 19, 20). Therefore, it has been postulated that in these instances Angiotonin may be the humoral agent responsible for the elevated arterial pressure. These considerations made it seem essential to determine whether the adminis-

tration of Angiotonin to normal subjects would produce arterial hypertension similar to that observed clinically in human cases.

## METHODS

The Angiotonin<sup>2</sup> was administered intravenously, either full strength in single injections ranging from 0.1 cc. to 2 cc., or as a 10 per cent solution (in saline) by constant infusion at rates up to 5 cc. per minute.

Arterial pressure was determined by the Hamilton manometer (21) in the femoral artery, or by the usual auscultatory method in the brachial artery. Venous pressure was measured in the antecubital vein either by the Hamilton manometer or by the method of Moritz and von Tabora (22). Pulse rate was counted from one of the several tracings obtained.

Temperature of the skin was measured by thermal junctions lightly attached to the skin by adhesive strips. Usually the pads of the fingers and toes, and occasionally points on the face, chest or forearms were selected for temperature measurements. Rectal temperature was determined by a thermal junction inserted at least 5 cm. above the internal rectal sphincter. The galvanometer allowed the detection of changes of 0.1° C. The room was draught-free and was maintained at a temperature as constant as possible.

Blood flow in the hand, forearm or calf was measured plethysmographically (23, 24). Measurements were made both in the normal resting condition, and also after full local vasodilatation had been induced by a 5-minute period of arterial occlusion (25). The latter type of measurements have been termed "reactive-hyperemia blood flows." Three minutes were allowed for recovery between each 5-minute period of arterial occlusion when such measurements were being made.

Circulation time was determined by the sodium cyanide method (26). Cardiac output was estimated in both the supine and erect positions on the ballistocardiographs of Dr. Isaac Starr (27), who very kindly made the records and did the calculations. Spinal fluid pressure, vital capacity, electrocardiograms, teleoroentgenograms and kymograms were obtained by the usual techniques. Dur-

<sup>2</sup> The Angiotonin was prepared and generously supplied by Dr. Irvine H. Page and his co-workers at the Lilly Laboratory for Clinical Research, Indianapolis, Indiana.

<sup>1</sup> Presented before The Society for Clinical Investigation at the annual meeting at Atlantic City, May 5, 1941.



TABLE I—Continued

R. C.	F	115	0.5	126/66	160/92	72	48	90	130										Hamilton recording.
E. C.	F	126	0.1 0.1 0.5	128/80 128/76 130/78	162/100 170/100 178/110			70 100 100	95 120 95										{ Spinal fluid pressure ( <i>mm.</i> ). Before, 175; after, 175. Before, 200; after, 215. Before, 230; after, 240.  ( Circulation time ( <i>seconds</i> ). Before, 25; after, 25.)
B. B.	F	111	0.1 0.5 0.5 1.0	110/60 122/66 128/66 126/68	138/70 160/84 160/88 180/94	66 66 72	60 54 60	25 60 50 60	70 125 85 110										
H. B.	M	131	0.1 2.0 2.0	120/70 124/70 120/72 120/70	134/84 148/110 170/110 172/114	81 75 80 72	72 80 75 69												
J. B.	M	135	0.1 0.5 1.0 0.25 0.5 1.0	115/64 114/60 114/62 125/75 122/80 128/74	125/75 130/84 140/98 146/98 160/110 190/140	77 80 81 103 111 115	75 63 60 97 94-111 103-125	60 60 75 55 60 60 60 95	70 85 120 60 80 95										Atropine 1.5 mgm. intravenously. Atropine 1.5 mgm. intravenously. Atropine 2.0 mgm. intravenously.
M. B.	F	135	0.1 0.5	138/74 130/74	150/80 180/92	72 66	66 54	60 50 125	80 125										
M. A.	M	150	0.1 0.5 1.0	112/74 110/70 120/74	134/84 166/110 182/110	78 78 75	66 60 68	90 100 140 95	100 140 140										
P. F.	M	131	0.5 1.0	134/78 138/74	160/98 172/110	92 94	75 75	53 50 93	81 93										
J. M.	F	104	0.5 0.5	125/64 132/76	150/95 196/120	71 115	52 100-130	60 50 90	90 140 140										Atropine 2 mgm. intravenously.
I. K.	M	158	2.0 2.0	126/70 112/80	150/98 152/104	72	68												Cardiac output.
T. C.	M	190	1.5	120/65 116/70	170/105 145/90	68 85	40 60												Cardiac output.
				125/70	140/90	74	67	103	135										Cardiac output.

\* Skin temperature of third finger.

\* Skin temperature of third finger.

Note: Twenty-five additional injections of amounts other than 0.1, 0.5, 1.0, 1.5, 2.0 cc. are omitted from the table for the sake of brevity.







TABLE II  
Intravenous infusions of 10 per cent solution of angiotonin

Name	Age	Sex	Weight lbs.	Infusion rate cc. per minute	Arterial pressure		Pulse		Venous pressure		Cold test arterial pressure				Blood flow				Skin temperature	
					Be-fore	Dur-ing	Be-fore	Dur-ing	Be-fore	Dur-ing	Before		During		Forearm or calf		Hand		Great toe	
											Control	Immer-sion	Control	Immer-sion	Be-fore	Dur-ing	Be-fore	Dur-ing	Be-fore	Dur-ing
					mm. Hg	mm. Hg	beats per minute	beats per minute	mm. Saline	mm. Saline	mm. Hg	mm. Hg	mm. Hg	mm. Hg	cc. per 100 cc. per minute	cc. per 100 cc. per minute	cc. per 100 cc. per minute	cc. per 100 cc. per minute	Degrees centigrade	Degrees centigrade
E. Z.	20	F.	130	2	114/74	138/100	78	72	60	155	114/70	130/100	138/100	144/116	2.5	3.5-1.5	13	19-3.9	21.0	20.0
E. W.	24	F.	125	1 2	100/60 100/60	120/86 160/104	78	51	60 60	100 150	108/64	110/60	120/86	130/84	1.4	1.6-1.2	18	18-2.5	33.2 33.2	31.0 30.0
H. S.	20	F.	115	3	116/64	168/110	72	60	95	160	120/60	124/84	164/110	172/114	1.8	1.8-1.7	9.9	12.4-3.8	31.5	30.0
N. P.	43	M.	150	3 4	124/74 124/74	148/90 170/104	90 72	66 72	60	160	124/74	148/90	148/90	168/106	2.5	3.6-2.5	15	17-7.0	19.5	19.5
A. M.	28	M.	145	2	108/60	136/94	78	66	135	150	106/64	118/50	130/90	140/110	3.5	2.5-3.3	21	15-24	17.5	17.5
V. M.	45	M.	150	1.5 2	114/70 114/70	144/94 166/106	72	60									38	29-42	34.0 34.0	33.0 31.0
D. M.	23	M.	161	4	124/68	146/100	84	66	80	160	126/74	134/84	146/100	166/110	2.8	7.0-3.5	37	52-10	31.0	27.5
A. M.	20	F.	120	5	104/64	150/100	80	73	65	170							25	23-1.9	17.5	17.0
W. L.†	29	M.	167	5	98/64	180/110	66	54	70	195					2.2	3.4-1.7	33	44-6.4	33.5	27.5
A. L.	55	M.	180	4	118/78	150/110	90	84	45	110	118/78	124/80	150/110	150/110	1.7	2.5	26	28-14	32.0	31.0
E. E.	30	F.	109	2	124/74	130/94	108	72	100	150	120/72	124/84	130/94	140/108	2.7	4.2-3.4	20	15-25	19.5	19.0
M. B.*	45	F.	135	3	124/70	184/94	66	48	80	120					2.0	6.3-2.0	3.7*	3.9-3.0	20.0	20.0
P. F.	40	M.	131	5	130/64	208/110			55	180										
T. C.	53	M.	190	4	128/75	185/108	72	63	95	170										

\* Circulation times: Before, 20 seconds; after, 23 seconds. Subject cool.

† Circulation times: Before, 18 seconds; after, 24 seconds.

ing the x-ray studies care was taken to prevent any movement of the subject in relation to the plates.

After all apparatus had been adjusted, at least half an hour was allowed to elapse before any Angiotonin was administered. Special precautions were taken to prevent the subject's knowing what was being given by substituting "blanks" of saline in cases of single injections or by changing from saline to Angiotonin, or *vice versa*, through a 3-way stop-cock during an intravenous infusion. Whenever an infusion apparatus was in place, as for measurements of venous pressure, single injections were made into the rubber tubing instead of directly into a vein. All venipunctures were rendered painless by antecedent novacainization.

The subjects were young adults without any evidence of cardiovascular disease (Tables I and II). They were studied usually in the recumbent position and were comfortably warmed by blankets and, if necessary, by electric heating pads. The warming was usually so regulated during the control period that vasodilatation occurred in the hands as shown by a temperature of 33° to 35° C. in the finger pads. The toes usually remained cool. This state represents one of moderate peripheral vasodilatation in which further vasodilatation would be apparent by an

increase in temperature of the toes, or vasoconstriction by a decrease in temperature of the fingers. The cold test of Hines and Brown (28) was done by immersing the subject's hand in iced water (4° C.) for 1 minute.

## RESULTS

Angiotonin was administered intravenously by single injection 150 times in 40 subjects (Table I) and by continuous infusion 14 times in 14 subjects (Table II). Some 15 to 30 seconds after an injection of Angiotonin, *arterial pressure* began to rise, and in about 2 minutes reached its peak, the height of which, in a given subject, was roughly proportional to the amount of Angiotonin injected. The systolic pressure rose somewhat more than the diastolic, resulting in an increase in pulse pressure (Figures 1, 2, 3). This increase in pulse pressure was always apparent in the records obtained with the Hamilton manometer (Figure 1). In some cases when the auscultatory

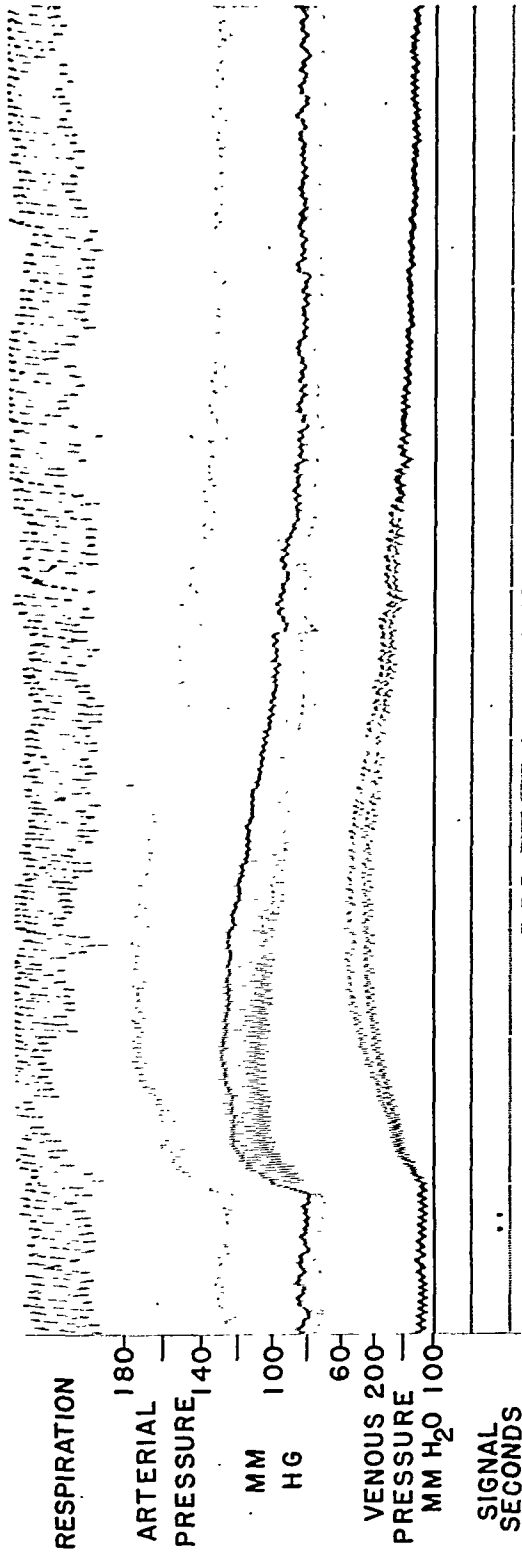


FIG. 1. EFFECTS OF AN INTRAVENOUS INJECTION OF ANGIOTONIN ON ARTERIAL PRESSURE AND VENOUS PRESSURE MEASURED IN A NORMAL SUBJECT (W. F.) WITH HAMILTON MANOMETERS

Respirations (inspiration on down stroke) above, and time marks (seconds and minutes) below. At the first signal the injection of 1.5 cc. of Angiotonin was begun, and at the second signal it was finished.

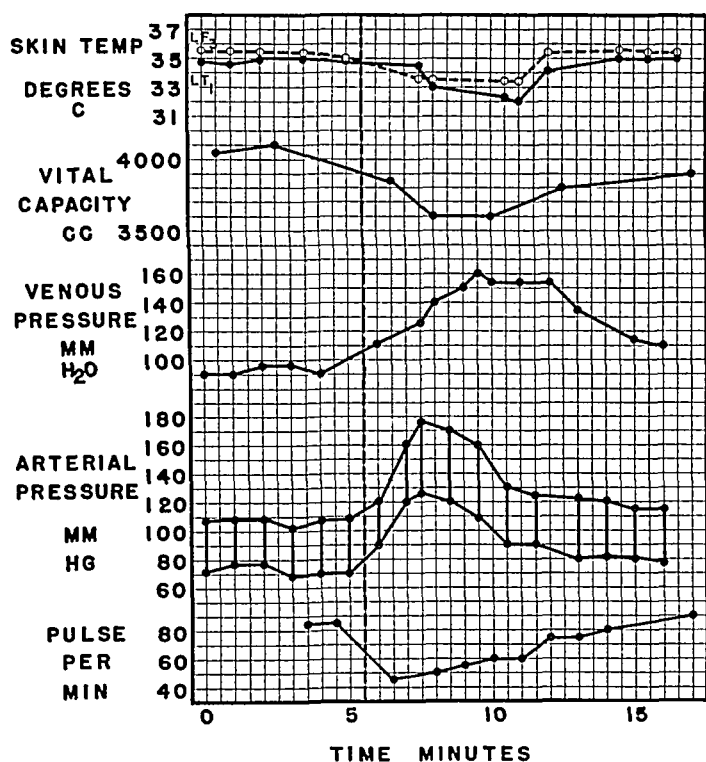


FIG. 2. EFFECTS OF AN INTRAVENOUS INJECTION OF ANGIOTONIN ON PULSE RATE, ARTERIAL PRESSURE, VENOUS PRESSURE, VITAL CAPACITY AND SKIN TEMPERATURE OF THE LEFT GREAT TOE (L T<sub>1</sub>) AND OF THE LEFT THIRD FINGER (L F<sub>3</sub>) OF A NORMAL SUBJECT (B. Z.)

At 5½ minutes (interrupted vertical line) 2 cc. of Angiotonin were administered.

method was used, it was difficult to determine accurately the associated systolic and diastolic pressures, because of the bradycardia and also because of a muffling of the first sounds coming past the cuff.

There was *bradycardia* (Tables I, II, Figures 1, 2, 3) with pulse rates often as low as 55 per minute. This bradycardia could be prevented by atropine. After atropinization (2 mgm. intravenously) Angiotonin did produce slight slowing of the pulse initially, but this was immediately followed by an acceleration of the pulse to or above its control level (Table I, Figure 4). Incidentally, the hypertensive effect of a given dose of Angiotonin was greatly enhanced after atropinization.

With the rise of arterial pressure after Angiotonin, *venous pressure* also rose, but more slowly, reaching its peak about 1 minute after the maximum rise in arterial pressure (Tables I, II, Figures 1, 2, 3). During the rise of venous pressure, the neck veins often became visibly more distended.

*Respiration* usually quickened slightly, but there was never any subjective sensation of dyspnea. However, the *vital capacity* decreased significantly (Tables I, II, Figures 2, 3).

*Rectal temperature* was never significantly affected by the administration of Angiotonin but the *temperature of the skin* usually decreased slightly (Tables I, II, Figures 2, 3). In many subjects definite pallor was apparent. Angiotonin when injected *intradermally* (0.1 cc.) in 5 subjects produced definite *local blanching*, but not so marked or widespread as that produced by a solution (1:1000) of epinephrine (0.1 cc.).

*Blood flow* in the limbs usually decreased moderately, but not always (Tables I, II). Characteristically, in the limbs the response was phasic, consisting of an initial increase in blood flow, followed by a decrease and finally a return to the control level (Figure 5). The changes in volume of the limbs always reflected the alterations in blood flow.

Some interest was attached to the nature of the initial increase in blood flow which occurred in the limbs after an intravenous injection of Angiotonin, since evidence was obtained that the *local action* of Angiotonin is vasoconstrictor: Minute amounts (up to 0.1 cc.) of Angiotonin injected directly into the *femoral artery* of 4 subjects caused only vasoconstriction locally in the calf (Figure 6). The initial vasodilatation after intravenous injection appeared simultaneously in the forearm and calf with the first rise of arterial pressure (Figure 5) and often before the expected arrival of the Angiotonin in the part, as calculated from the circulation time. The appearance and amplitude of this increase in blood flow were not directly related to the size of the dose of Angiotonin or to the height to which the arterial pressure subsequently rose. These facts suggested that this initial vasodilatation was due to some mechanism other than the local action of Angiotonin. That it probably was the result of sympathetic vasomotor activity was demonstrated in a subject with a unilateral (right) cervical sympathectomy performed 9 years previously for epilepsy. In this subject the intravenous administration of 0.15 cc. of Angiotonin produced the initial vasodilatation in the normally innervated forearm, but only vasoconstriction in the sympathectomized forearm (Figure 7).

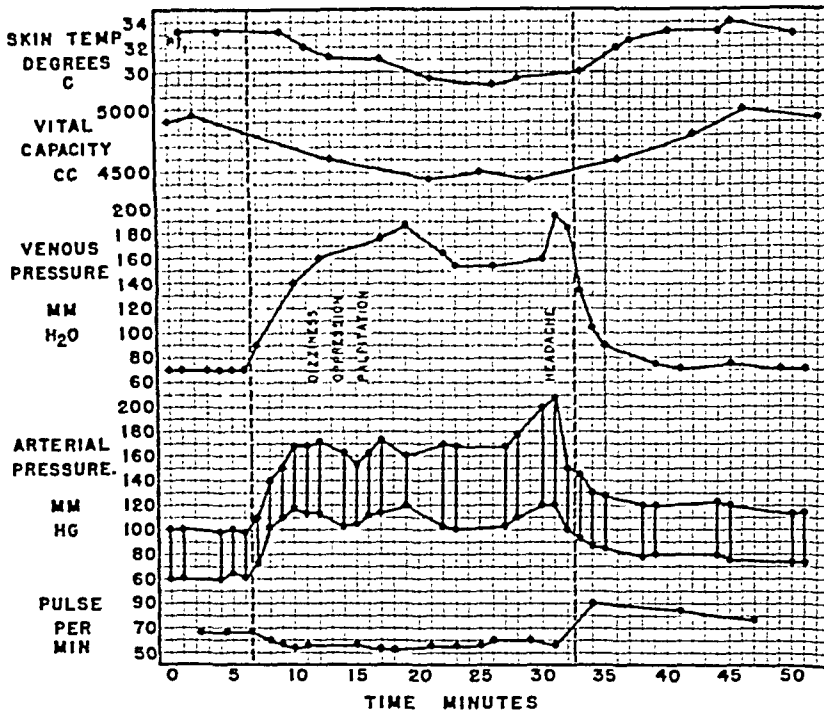
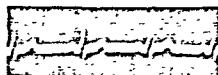


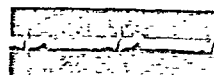
FIG. 3. EFFECTS OF AN INTRAVENOUS INFUSION OF ANGIOTONIN ON PULSE RATE, ARTERIAL PRESSURE, VENOUS PRESSURE, VITAL CAPACITY, AND SKIN TEMPERATURE OF THE RIGHT GREAT TOE OF A NORMAL SUBJECT (W. L.)

At 6½ minutes (first interrupted vertical line) the infusion of a 10 per cent solution of Angiotonin was begun, at a rate of 5 cc. per minute. Symptoms noted by the subject are recorded. At 32½ minutes (second interrupted vertical line) the infusion of Angiotonin was ended.

#### BEFORE ATROPINE

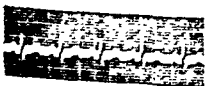


CONTROL  
PULSE RATE 71  
ARTERIAL PRESSURE 126/64  
VENOUS PRESSURE 55

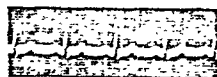


1 MIN AFTER ANGIOTONIN  
PULSE RATE 52  
ARTERIAL PRESSURE 120/75  
VENOUS PRESSURE 80

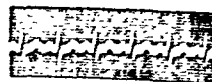
#### AFTER ATROPINE 2 MG INTRAVENOUSLY



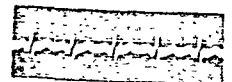
CONTROL  
PULSE RATE 115  
ARTERIAL PRESSURE 132/76  
VENOUS PRESSURE 50



1 MIN AFTER ANGIOTONIN  
PULSE RATE 100  
ARTERIAL PRESSURE 185/120  
VENOUS PRESSURE 90



3 MIN AFTER ANGIOTONIN  
PULSE RATE 130  
ARTERIAL PRESSURE 192/105  
VENOUS PRESSURE 95



14 MIN AFTER ANGIOTONIN  
PULSE RATE 115  
ARTERIAL PRESSURE 136/86  
VENOUS PRESSURE 55

FIG. 4. EFFECTS OF AN INTRAVENOUS INJECTION OF ANGIOTONIN (0.5 CC.) ON THE ELECTROCARDIOGRAM (LEAD 1) OF A NORMAL SUBJECT (P. F.), BEFORE AND AFTER ATROPINIZATION (2 MGM. ATROPINE INTRAVENOUSLY)

Spinal fluid pressure measured either in the lumbar or cisternal space of 5 subjects was not significantly altered by injections of Angiotonin which caused marked rises of arterial pressure

(Table I). A small rise (20 mm. H<sub>2</sub>O) in spinal fluid pressure usually occurred, but this amount of change, or more, could be produced by questioning the subject, or by his changing his re-

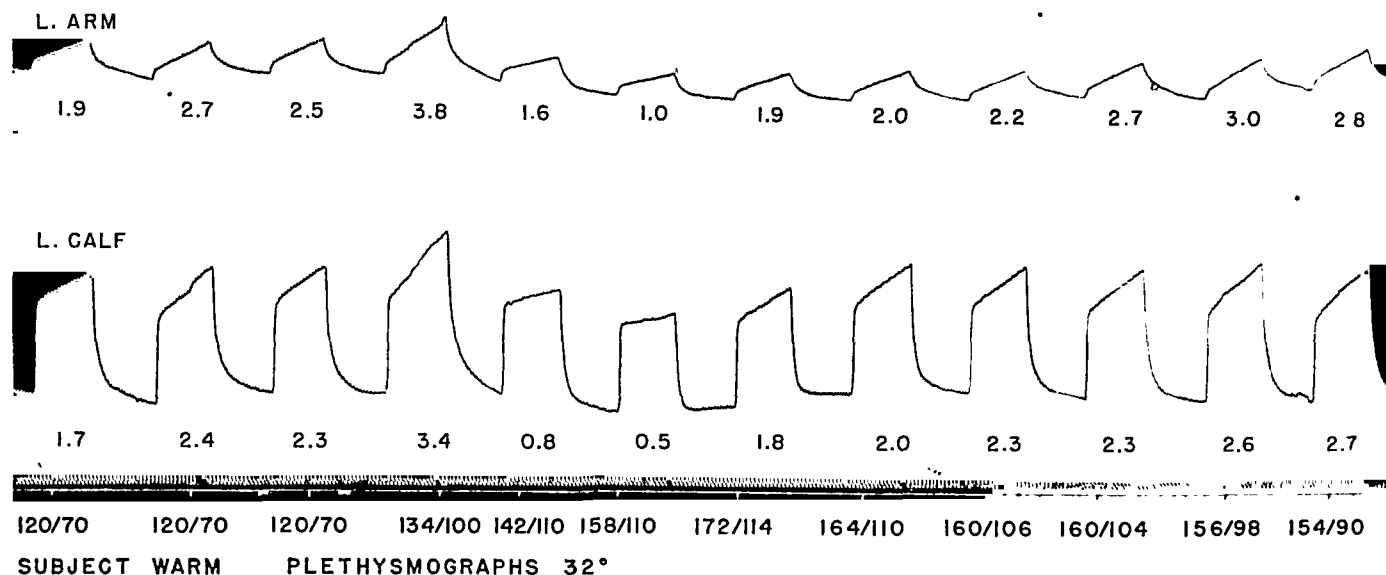


FIG. 5. EFFECTS OF AN INTRAVENOUS INJECTION OF ANGIOTONIN ON THE PLETHYSMOGRAPHIC TRACINGS OF BLOOD FLOW TO THE LEFT FOREARM AND LEFT CALF OF A NORMAL SUBJECT (H. B.)

The first sharp rise of each tracing of blood flow (especially marked in the calf) is due to the displacement of tissue into the plethysmograph by inflation of the proximal cuff. The true blood flow is indicated by the steepness of the straight portion of the curve following the initial sharp rise. The figures under each tracing of blood flow give its calculated value in cc. per 100 cc. per minute. Time: seconds. Signals: single, for measurement of arterial pressure, given below in mm. Hg; multiple, for beginning and end of an intravenous injection of 2 cc. of Angiotonin.

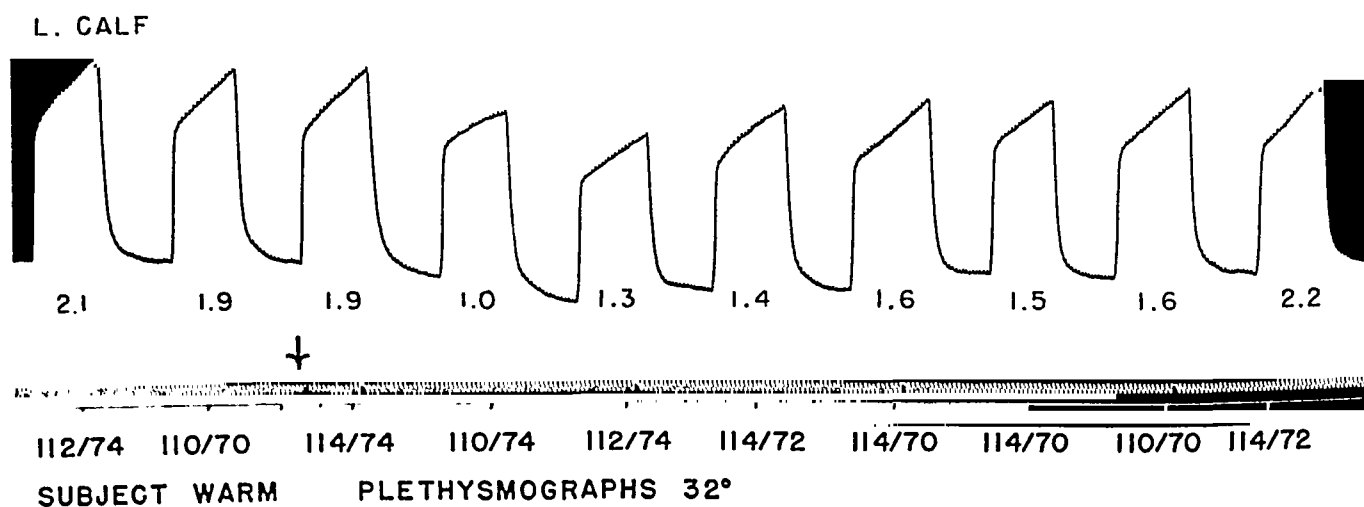


FIG. 6. EFFECTS OF AN INTRA-ARTERIAL INJECTION OF ANGIOTONIN ON THE PLETHYSMOGRAPHIC TRACINGS OF BLOOD FLOW TO THE LEFT CALF OF A NORMAL SUBJECT (D. S.)

The figures under each tracing of blood flow give its calculated value in cc. per 100 cc. per minute. Time: seconds. Signals: short, for measurement of arterial pressure given below in mm. Hg; long (arrow), for an injection of 0.4 cc. 10 per cent Angiotonin into the left femoral artery.

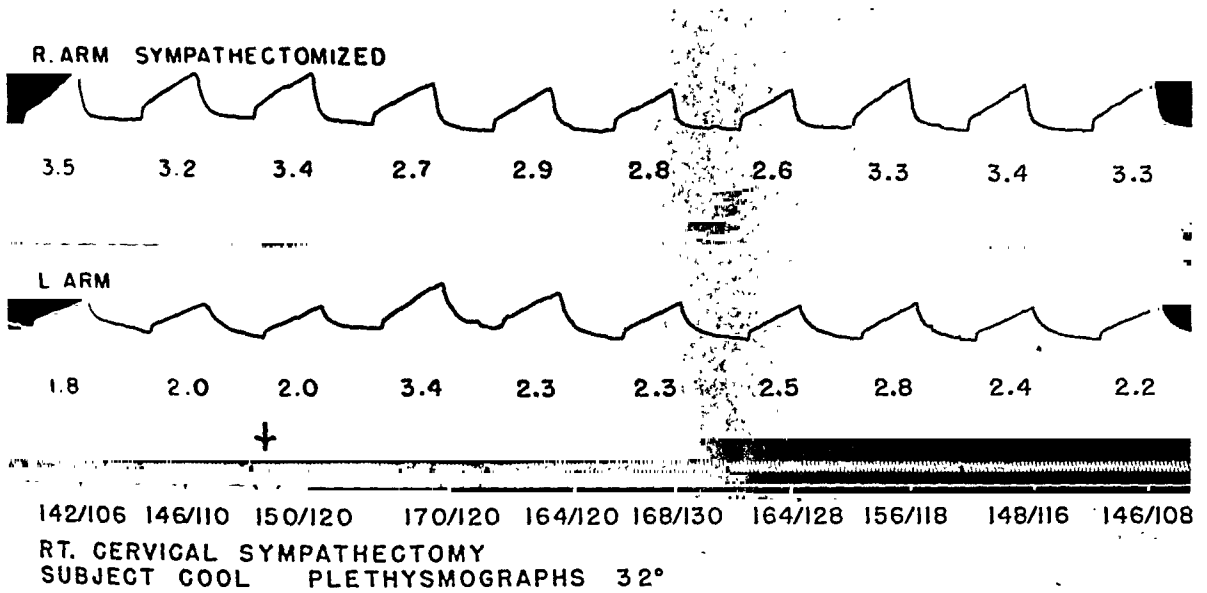


FIG. 7. EFFECTS OF AN INTRAVENOUS INJECTION OF ANGIOTONIN ON THE PLETHYSMOGRAPHIC TRACINGS OF BLOOD FLOW TO THE LEFT FOREARM AND RIGHT FOREARM OF A SUBJECT (L. R.) WHO HAD SUSTAINED A RIGHT CERVICAL SYMPATHECTOMY 9 YEARS PREVIOUSLY FOR EPILEPSY

The figures under each tracing of blood flow give its calculated value in cc. per 100 cc. per minute. Time: seconds. Signals: short, for measurement of arterial pressure, given below in mm. Hg; long (arrow), for an intravenous injection of 0.15 cc. Angiotonin.

spiratory rate slightly. It was therefore not considered significant.

No differences were found in the action of Angiotonin given by single injection and by continuous intravenous infusion (Table II) except for the duration of the effects which could be prolonged apparently unchanged for periods up to three quarters of an hour (Figures 3, 8, 9). No experiments were carried on longer than this, mainly because of the scarcity of the Angiotonin. When the rate of an infusion was increased, there was a further rise in arterial pressure (Table II, Figure 8). Thus by varying the rate of administration, any desired level of arterial pressure could be maintained. The other circulatory effects produced by Angiotonin also continued at stable levels as long as a constant infusion was given.

During the hypertension due to Angiotonin mild symptoms of dizziness, substernal oppression, palpitation, nausea or headache were occasionally noted. None of these was severe, except in one instance during an infusion when a headache accompanied a sharp further rise in arterial pressure (Figure 3). This sharp increase was

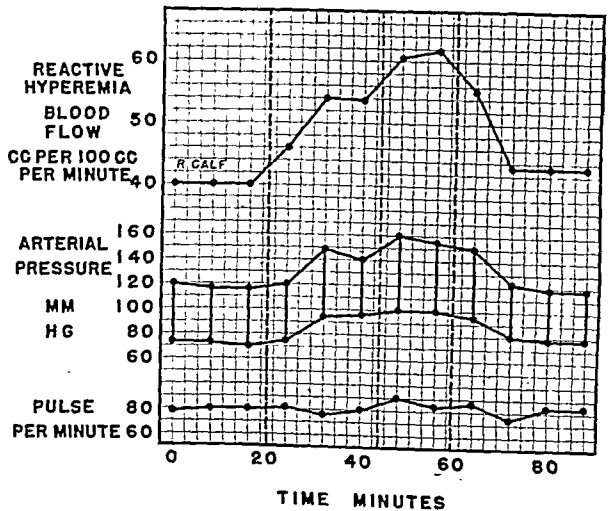


FIG. 8. EFFECTS OF AN INTRAVENOUS INFUSION OF ANGIOTONIN ON THE PULSE RATE, ARTERIAL PRESSURE AND REACTIVE-HYPEREMIA BLOOD FLOW IN THE RIGHT CALF OF A NORMAL SUBJECT (V. M.)

At 20 minutes (first interrupted vertical line) an intravenous infusion of 10 per cent Angiotonin was started at a rate of 1.5 cc. per minute. At 44 minutes (second interrupted vertical line) the rate of infusion was increased to 2 cc. per minute. At 60 minutes (third interrupted vertical line) the infusion of Angiotonin was ended.

thought to be due to the fact that just previously the subject had made a particularly strenuous expiratory effort for the test of vital capacity, probably holding some of the solution back in the vein. With the subsequent deep inspiration this was quickly drawn into the general circulation, producing the sudden further rise. At any rate, the infusion was stopped, and the headache and hypertension quickly subsided.

During an infusion of Angiotonin the *blood flow* in the limbs remained under the control of the sympathetic nervous system. Vasoconstrictions and vasodilatations known to be mediated by the sympathetic nervous system (24, 29) took place as before in response to stimuli. For example, vasodilatation in the feet, in response to

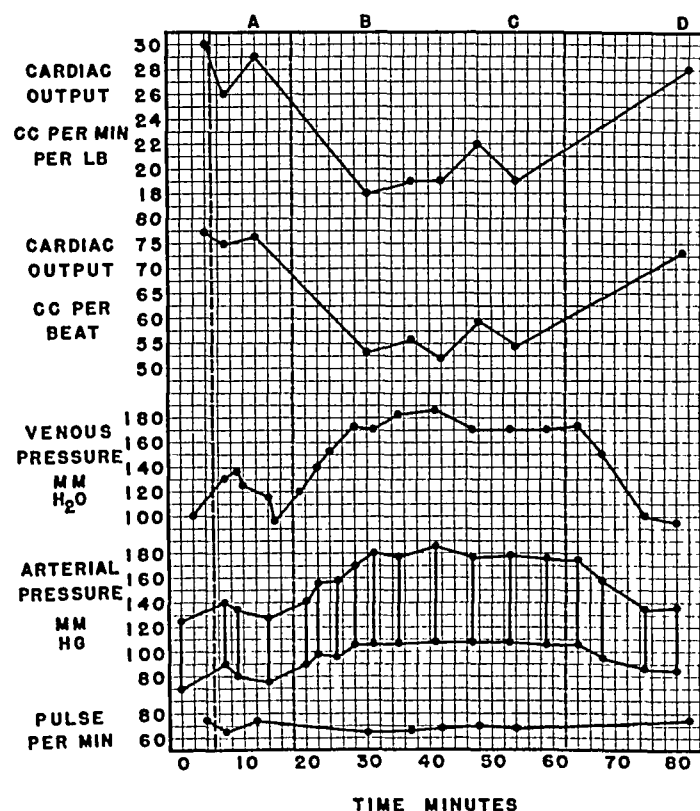


FIG. 9. EFFECTS OF ANGIOTONIN ON THE PULSE RATE, ARTERIAL PRESSURE, VENOUS PRESSURE AND CARDIAC OUTPUT (PER BEAT AND PER MINUTE) OF A PATIENT (L. C.) WITH ECZEMA

At 5 minutes (first interrupted vertical line) a single intravenous injection of 1.5 cc. Angiotonin was given. At 18 minutes (second interrupted vertical line) an intravenous infusion of 10 per cent Angiotonin was started at a rate of 4 cc. per minute. At 62 minutes (third interrupted vertical line) the infusion of Angiotonin was ended. The letters at the top of the chart refer to representative ballistocardiographic tracings shown in Figure 10.

TABLE III  
*Reactive-hyperemia blood flow*

Name	Amount of angiotonin	Arterial pressure		Blood flow	
		Control	After	Control	After
SINGLE INJECTIONS					
	<i>cc.</i>	<i>mm. Hg</i>	<i>mm. Hg</i>	<i>cc. per 100 cc. per minute</i>	<i>cc. per 100 cc. per minute</i>
E. T.	1.0	120/70	146/90	32	44
D. M.	1.0	120/64	144/90	32	48
M. A.	1.0	114/74	182/110	18	33
H. B.	0.1	118/70	124/78	28	32
	0.1*	118/70*	124/78*	34*	34*
	1.0	120/74	138/100	27	41
	1.0*	120/74*	138/100*	25*	45*
	2.0	114/70	150/114	24	43
	2.0*	114/70*	150/114*	28*	51*

INFUSIONS					
E. E.	cc. per minute 2	114/68	140/100	20	28
A. L.	4	124/82	156/100	21	28
A. M.	4	108/68	136/98	25	31
V. M.	1.5	114/70	148/94	31	54
	2		160/100	31	61
A. M.	5	108/64	130/84	13	21
N. P.	4	120/74	166/98	18	25
H. S.	3	120/60	148/100	23	28
E. Z.	2	116/64	146/104	16	29

\* = Left arm. Other flows = Left leg.

strong warming of the body, if begun during the control period, continued (after a slight pause) when Angiotonin was administered. Likewise, vasoconstriction in response to the cold test of Hines and Brown took place during an infusion of Angiotonin, as before. It is of considerable interest that the *pressor response* to the cold test of Hines and Brown was not significantly altered in 8 normal subjects during the hypertension produced by an infusion of Angiotonin (Table II).

The *reactive-hyperemia blood flow*, in the forearm or calf (during full local vasodilatation produced by a 5-minute period of arterial occlusion), increased with the rise of arterial pressure due to Angiotonin, and decreased when the arterial hy-

TABLE IV  
*Cardiac output*

Name	Age	Sex	Height	Weight	Amount of angiotonin	Arterial pressure			Pulse rate			Cardiac output			Cardiac output		
						Control	Angio-tonin	Re-cov-ery	Control	Angio-tonin	Re-cov-ery	Control	Angio-tonin	Re-cov-ery	Control	Angio-tonin	Re-cov-ery
HORIZONTAL POSITION																	
J. M.	32	F	4 11	104	0.5	121/78	150/98	124/78	72	68	78	33	24	34	23	16	25
R. W.	34	M	5 6½	150	1.0	120/80	160/110	118/82	70	52	72	49	46	45	23	16	22
H. K.	26	M	5 8½	158	2.0	121/65	170/105	122/68	68	40	66	58	51	57	25	13	24
T. C.	53	M	5 11½	190	1.5	125/70	140/90	135/80	74	67	72	77	75	76	30	26	29
T. C.	53	M	5 11½	190	4 cc. per minute*	128/75	175/108	136/84	72	63	72	76	53	73	29	18	28
VERTICAL POSITION																	
R. W.	34	M	5 6½	150	1.0	120/100	148/120	125/100	82	68	76	43	40	43	23	18	22
H. K.	26	M	5 8½	158	2.0	116/70	145/90	115/68	85	60	80	42	35	39	23	13	20

\* Infusion of 10 per cent solution of angiotonin.

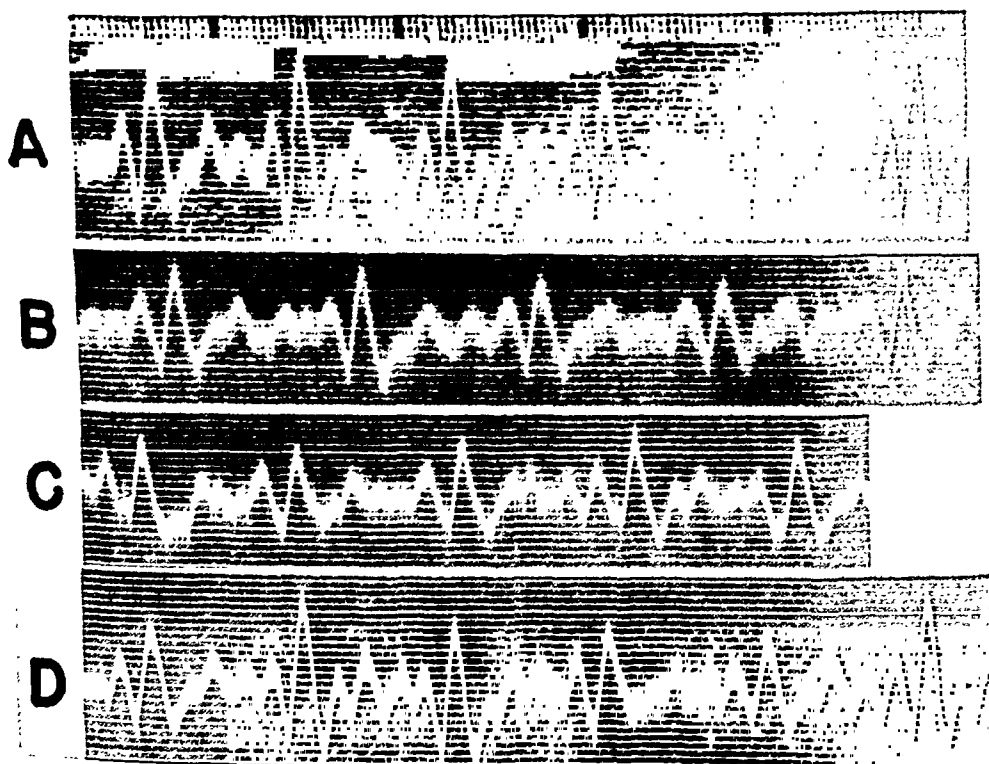


FIG. 10. REPRESENTATIVE BALLISTOCARDIOGRAPHIC TRACINGS OBTAINED IN THE EXPERIMENT SHOWN IN FIGURE 9

The letters correspond to the times indicated at the top of Figure 9.



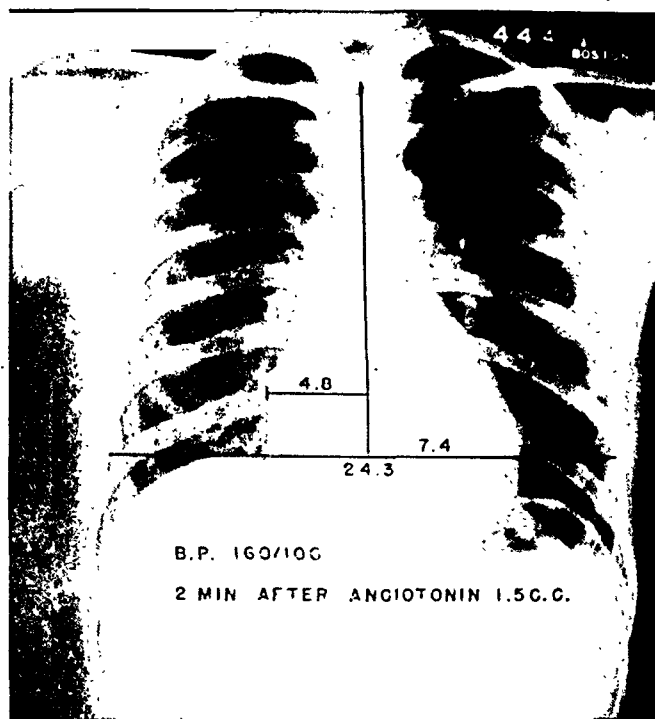
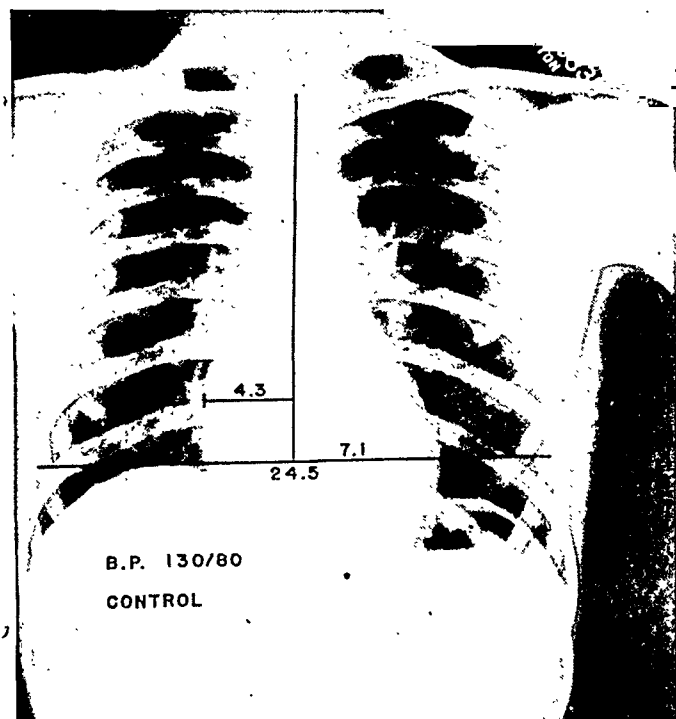


FIG. 11. EFFECTS OF AN INTRAVENOUS INJECTION OF ANGIOTONIN (1.5 CC.) ON THE TELEOROENTGENOGRAMS OF A NORMAL MALE (W. F.)

Cardiac measurements are given in cm. Arterial pressures recorded below in mm. Hg were taken immediately before the teleoroentgenograms.

pertension subsided after the infusion of Angiotonin was stopped (Table III, Figure 8).

The *cardiac output* (blood flow to the entire body) measured ballistocardiographically in the supine and upright positions decreased after Angiotonin, both when calculated as cc. per minute per pound, and as cc. per beat<sup>3</sup> (Table IV). In one subject (Figure 9) after a single injection of Angiotonin, with a moderate rise of arterial and venous pressures, the change in output per beat was negligible, probably because of the bradycardia. However, during an infusion of Angiotonin, which resulted in a prolonged marked rise of arterial and venous pressures, there was a profound decrease in the cardiac output per beat as well as per minute (Figures 9, 10).

The arm-to-carotid sinus *circulation time* usually increased, often only slightly, but occasionally considerably (Tables I, II).

The *electrocardiograms* showed no significant changes other than the bradycardia (Figure 4).

*X-ray examinations* of the heart showed in 6 of 8 subjects a small but definite increase in the

cardiac size (Table V, Figure 11). Kymograms usually showed a slight decrease in the amplitude of the cardiac pulsations. In some subjects, in addition to the increase in the cardiac shadow, there was also an increase in the pulmonary markings.

The effects of Angiotonin administered intravenously subsided gradually in 4 to 10 minutes after the cessation of the administration, whether by continuous infusion or by single injection.

TABLE V  
*Teleoroentgenograms of heart*

Name	Amount of angiotonin	Arterial pressure		Transverse diameter of heart		Longitudinal diameter of heart		Cardiac axis	
		Control	After	Control	After	Control	After	Control	After
	cc.	mm. Hg		cm.		cm.		degrees	
R. M.	1.5	130/80	196/118	13.2	14.4	15.0	14.8	52	56
W. L.	2.0	114/80	170/110	12.3	12.2	14.0	14.2	50	45
C. R.	2.0	134/84	184/120	13.4	14.0	14.4	15.2	48	47
R. W.	1.2	130/84	174/134	13.9	14.0	14.9	14.9	65	68
B. Z.	1.5	120/70	158/110	12.8	13.5	13.6	14.0	56	55
C. D.	1.5	126/70	160/124	13.5	13.9	14.1	15.0	55	50
W. F.	1.5	130/84	164/110	11.7	12.3	13.5	14.4	45	40
P. F.	5 cc. per minute*	130/66	191/100	13.5	14.8	15.0	15.9	52	54
Average		126/78	178/116	13.0	13.6	14.3	14.8		

\* Infusion of 10 per cent solution of angiotonin.

<sup>3</sup> So calculated for the sake of comparison. The absolute values may, with the newer knowledge of the ballistocardiogram, prove slightly inaccurate quantitatively.

## DISCUSSION

The most interesting aspect of the arterial hypertension produced by Angiotonin is the associated evidence of "myocardial failure" occurring in otherwise normal individuals. An increase in venous pressure, decrease in vital capacity, increase in cardiac size, decrease in cardiac output and increase in circulation time, all important signs of heart failure, were frequently found. The mode by which Angiotonin could produce *heart failure* is difficult to explain, unless it exerts a direct "toxic" action on the myocardium. The experimental evidence in animals is against this explanation. On the contrary, Angiotonin seems to exert a stimulating or "tonic" effect on the myocardium (isolated heart), increasing its output and efficiency (29, 30).

It would appear that in man Angiotonin produces marked and generalized peripheral vasoconstriction, since it causes not only a rise in arterial pressure but also a decrease in cardiac output. The effects on cardiac output here reported are in essential agreement with those of Bradley and Parker (32). Likewise, the blood flow in every area in which measurements have been made, including the renal (33, 34), has been found to decrease after Angiotonin, at least initially. The peripheral vasoconstriction must be primarily on the arterial (arteriolar) side of the circulation since the rise of arterial pressure precedes the increase in venous pressure and the other signs of "myocardial failure."

Except for the rise in venous pressure and the decrease in cardiac output, the circulatory effects of Angiotonin, though definite when compared with the control measurements of the same subject, usually are not so great as to be clearly abnormal. Hence the increase in venous pressure and decrease in cardiac output can be said to be the outstanding characteristics of the Angiotonin hypertension.

It is difficult to judge whether naturally occurring hypertension is similar to the hypertension due to Angiotonin. However, one can state that, as commonly accepted, essential hypertension is *not* entirely similar to the hypertension of Angiotonin. The chief difference is the absence of a significant elevation of venous pressure such as was observed after Angiotonin. On the other

hand, as Starr has pointed out, a decrease in *cardiac output*, measured ballistocardiographically, is found in a considerable number of patients with essential hypertension (34). Furthermore, the amount of the decrease is about the same as that produced by Angiotonin (35). A possible explanation for the absence of the increased venous pressure and the other signs of "myocardial failure" in established cases of essential hypertension is that such signs may have been present initially, only to disappear after sufficient time had elapsed for the adjustments of "compensation" to take place.

One form of naturally occurring hypertension, namely that associated with acute hemorrhagic nephritis, does seem to be similar to the hypertension of Angiotonin. Both are characterized by an elevation of venous pressure and by x-ray evidence of increases in cardiac size and pulmonary congestion (36). These similarities may be apparent not only because of the sudden onset and acute nature of the two types, but also because of the availability of control or recovery measurements in the same individuals for comparison.

The blood flow in the limbs after the administration of Angiotonin tends to decrease, but remains under the control of the sympathetic nervous system. The resting blood flow, after an initial phasic variation, may be slightly, or even considerably, below the control level, but sympathetic vasodilatation and vasoconstriction can occur as before in response to appropriate stimuli. In this respect the hypertension due to Angiotonin is compatible with essential hypertension. The variable amount of the increase in circulation time in different individuals after Angiotonin could be explained entirely on the variable amount of associated decrease in the peripheral blood flow (37).

That Angiotonin produces local vasoconstriction is established not only from the evidence already cited, but also by the observations on its vasoconstrictor effects when injected intradermally or intra-arterially. But it is important that the peripheral vasoconstriction caused by Angiotonin can be released by physiologic procedures producing vasodilatation. Thus the *reactive-hyperemia* blood flows obtained during full local

vasodilatation after a 5-minute period of arterial occlusion increased along with the arterial pressure. This demonstrates that there is no danger of an inadequate blood flow with tissue anoxia in the extremities during the action of Angiotonin. On the contrary, because of the higher arterial pressure, the blood flow, if needed, can actually be greater than normal.

These observations on the reactive-hyperemia blood flow during the hypertension produced by Angiotonin are similar to those obtained during other pressor procedures (38). They emphasize the direct relationship which exists in a given individual between arterial pressure and blood flow in the limbs under controlled vasodilatation. But again, because of the wide scattering of the values for blood flow obtained in different individuals, whether normal or hypertensive, such measurements are of questionable value in estimating the degree and nature of the peripheral resistance to blood flow in a random subject or in two groups of individuals. It can be stated, however, that the reactive-hyperemia blood flows obtained during the hypertension due to Angiotonin are comparable with those obtained in young individuals with naturally occurring hypertension. Therefore, they are consistent with the conclusion that the increased peripheral resistance to blood flow in such hypertensive patients also can be released by similar physiologic vasodilatation.

The prevention of bradycardia after Angiotonin by atropine would indicate that the cardiac slowing is probably vagal in origin. Furthermore, the fact that the hypertensive effect of a given dose of Angiotonin is enhanced after atropine shows that the bradycardia is an important moderator mechanism. Probably another such moderator mechanism, though less effective, is the initial sympathetic vasodilatation in the limbs after Angiotonin.

It was surprising to find that no significant rise in spinal fluid pressure occurred after Angiotonin, in spite of considerable increases of arterial and venous pressures. This was interpreted as indicating that after Angiotonin a marked vasoconstriction occurs in the small vessels of the cerebrospinal contents, with a probable decrease in blood flow. This change was thought to be analogous to the decrease in volume and blood flow which may occur in the limbs after Angiotonin

along with a rise of arterial and venous pressures.

The finding of no significant difference in the pressor response to the cold test of Hines and Brown in the same subject when normal, and when hypertensive from Angiotonin, is of considerable interest. It casts doubt upon the usual explanation of why there should be a hyper-reactor response in hypertensive, or potentially hypertensive, individuals. This explanation postulates a greater amount of sympathetic vasoconstrictor "reserve" in such persons, due to a state of relative sympathetic vasodilatation which, in turn, is a moderator response to the presence of a circulating pressor substance. This greater reserve is called into play by a strong vasoconstrictor stimulus, such as the cold test, giving a "hyper-reactor" response. The experiments here reported would indicate that some other factor, possibly hereditary, is responsible for the hyper-reactivity of the sympathetic nervous system in certain individuals, and that such hyper-reactivity is not necessarily imposed upon a normal sympathetic nervous system by incipient or actual hypertension.

The symptoms produced by Angiotonin were usually mild, but somewhat proportional to the dosage, and to the resultant rise in arterial pressure. They resembled the complaints of patients with clinical hypertension, and help to identify such complaints as arising in actual pathologic physiology instead of being attributable to "psychoneurosis."

#### SUMMARY AND CONCLUSIONS

1. Angiotonin administered intravenously produces in normal subjects arterial hypertension which can be controlled by regulating the rate of administration.

2. This arterial hypertension is accompanied by an increase of venous pressure, and frequently by other signs of "myocardial failure" including:

- a. Decrease in vital capacity
- b. Increase in circulation time
- c. Decrease in cardiac output
- d. Increase in cardiac size

3. There is bradycardia, probably vagal in origin.

4. Spinal fluid pressure is not significantly altered.

5. The electrocardiogram reveals no important changes except bradycardia.

6. The temperature of the skin usually decreases, but remains responsive to alterations of body temperature.

7. Blood flow measured plethysmographically in the limbs tends to decrease but remains under the control of the sympathetic nervous system.

8. Reactive-hyperemia blood flow (measured during full local vasodilatation produced by a 5-minute period of arterial occlusion) increases with the rise of arterial pressure.

9. The pressor response to the cold test of Hines and Brown is not altered during the hypertension.

10. Mild symptoms of dizziness, substernal oppression, headache, nausea or palpitation may be noted.

11. The effects subside 4 to 10 minutes after the cessation of administration, whether by single injection or by continuous infusion.

12. Injected intradermally, Angiotonin produces local blanching of the skin.

13. Injected intra-arterially, it produces vasoconstriction in the muscular parts supplied by the artery.

This investigation was conducted with the technical assistance of Miss Sara B. Merritt, B.S.

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# SOME PROPERTIES OF HUMAN FETAL AND MATERNAL BLOOD

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The differences between the bloods of pregnant animals and of their fetuses, particularly in regard to the properties of the hemoglobin, have interested workers for many years. However, the results of different workers are in conflict on several points, perhaps because of species differences, or of varying conditions for obtaining blood or differing methods of calculation.

The earliest evidence of difference between fetal and maternal hemoglobin was the greater resistance to alkaline denaturation observed in the fetal hemoglobin (1, 2, 3). These workers calculated that adult blood contained up to 20 per cent of the alkaline-resistant hemoglobin, whereas fetal blood consisted almost wholly of the resistant type. Differences in crystal structure (2, 3) have added to the evidence for difference in the hemoglobins.

The property of fetal and maternal blood on which the greatest amount of work has been done is the oxygen affinity. This property is usually described by an oxygen dissociation curve which presents graphically the amount of oxygen bound to hemoglobin through a range of oxygen pressures. Plotted on the usual arithmetic coordinates with oxygen pressures as abscissae and % HbO<sub>2</sub> as ordinates, the curve has the well known S-shape. The shape of the curve has been found to be quite constant among adult animals of a given species but to vary considerably from species to species. It has been noted that various conditions displace the curve to right or left without changing the fundamental shape. Among these the most important is the pH. In a more acid medium the hemoglobin has less affinity for oxygen at all pressures and its curve is therefore shifted to the right; conversely, a more alkaline reaction shifts it to the left.

The oxygen dissociation curve is frequently plotted on a logarithmic scale, in which case the

characteristics of shape and lateral displacement are more easily dissociated. Plotting  $\log \frac{100 \text{ Hb}}{\text{HbO}_2}$  against  $\log p\text{O}_2$ , the curve has been found to approximate a straight line, the slope of which has the same significance as the shape observed in the arithmetic curve and of which the intercept of either axis measures the lateral displacement. The Hill-Barcroft empirical equation describes mathematically this type of relationship; so, from the logarithmic graph of a given blood the constants of the equation can be easily determined and used to describe the characteristics of the dissociation curve. The usual form of the equation is

$$y = 100 \left( \frac{Kx^n}{1 + Kx^n} \right)$$

where  $y = \% \text{ HbO}_2$ ,  $x = p\text{O}_2$  and  $K$  and  $n$  are constants. Transforming this equation into the units of the logarithmic graph, one obtains:

$$\log \frac{100 \text{ Hb}}{\text{HbO}_2} = -n \log x - \log K + 2.$$

From this it will be seen that the slope of the graph is  $-n$  and that  $K$  is easily calculated from any one point (e.g.  $\text{Hb} = \text{HbO}_2$ ).

With this brief discussion as a background, we may review the evidence in the literature on the comparison of the oxygen dissociation curves of fetal and maternal hemoglobin. Among the first investigations was Huggett's demonstration (4) that diffusion alone accounts for the gas transfer across the placenta in the goat. Incidentally, it appeared from his data that the fetal curve lay to the right of the maternal, i.e., at any tension the fetal blood combined with less oxygen than the maternal. This rather unfavorable physiological situation has not been confirmed in any species of animal. In fact, almost all investigations on humans (5, 6, 7) have found the opposite to be true, namely that the fetal oxygen disso-

ciation curve lay to the left of the maternal. Since Haselhorst and Stromberger worked with curves constructed from a single point according to the Hill-Barcroft equation, nothing could be said about differences in the shape of the curve. However, Eastman *et al.* found a different shape in the fetal blood curve in all their cases and Leibson *et al.* found a characteristic shape to the fetal blood curves in several of theirs. The difference in shape consisted in a greater shift to the left in the lower range than in the upper. On the other hand, Noguchi (8) constructed his curves on a logarithmic scale to determine the constants of the Hill-Barcroft equation, and concluded that the constant "*n*," which measures the slope of the logarithmic curve, had similar values in fetal and normal bloods.

Among the other animals studied have been the goat (9, 10), the rabbit (10), the chick (11), and the calf (12). In all, the curve for fetal blood was found to lie to the left of that of pregnant and non-pregnant adult animals. In the goat (9) it had an abnormal shape as well as a general displacement to the left. Furthermore, both these changes were found to be similar in buffered hemoglobin solutions (13). The latter finding is in contrast to the paradoxical report by Haurowitz (3) that the curve was shifted to the right for solutions of human fetal hemoglobin but to the left for cells suspended in saline or plasma. Apparently this paradox has not been confirmed.

Differences between blood of pregnant and non-pregnant animals have in almost all cases been observed as small and attributable to differences in pH. Frequently the curves were obtained at a constant  $p\text{CO}_2$  (e.g. 40 mm. Hg) in the equilibrating gas. Under this condition the pH of two bloods will approximate the same standard only provided the buffer power (i.e. the bicarbonate) of the bloods is equal. For example, the blood of a pregnant animal with a lower bicarbonate will be more acid at constant  $p\text{CO}_2$  than that of the non-pregnant animal used for comparison. Measured at constant  $p\text{CO}_2$ , according to Leibson *et al.* (7), the human maternal curve is to the right of the non-pregnant normal; calculated to constant pH, they found the maternal curves lay slightly to the left. Other workers have calculated their curves either at constant

$p\text{CO}_2$  or at the  $p\text{CO}_2$  of the blood as taken. It is impossible from their data to recalculate their curves to a given hydrogen ion concentration. Whereas it is the consensus of opinion that deviations in the maternal curves are attributable to differences in pH, the considerable magnitude of the shift in fetal blood has been taken to indicate the existence of a special fetal type of hemoglobin.

The primary purpose of this work is to acquire sufficient data on the bloods of non-pregnant and of pregnant women and of the fetuses at term to determine the complete oxygen dissociation curves at constant pH. The importance of choosing a standard pH rather than a standard  $p\text{CO}_2$  is apparent from the previous discussion, especially in order to investigate the fundamental question of a special fetal hemoglobin. To correct our curves to constant pH it was necessary to measure the bicarbonate content, calculate the pH, and establish the degree of shift in the oxygen dissociation curve with changes in pH. Likewise, because of the conditions of labor and anesthesia, the blood lactic acid values were determined in order to be able to extrapolate the acid-base conditions back to the period before the onset of labor.

Since Barcroft (9) has emphasized that in goats the fetal type of hemoglobin is most pronounced at eighteen weeks of gestation and has disappeared at birth or soon thereafter, we have attempted to determine curves of human blood before and after the normal ninth month of development. To do this we obtained bloods of infants born prematurely, and in two instances of infants born at term we obtained bloods periodically throughout the first month of life. So that changes might similarly be plotted for the mother, maternal bloods were studied throughout the last half of pregnancy.

In so far as sufficient blood was available, determinations of sodium, chloride, total base and total nitrogen were made on the serum. In this way a gross electrolyte change could be detected if it were sufficient to cause shift in the oxygen dissociation curve.

It is hoped that the sum total of evidence can be integrated to answer the remaining questions regarding the properties of so-called fetal hemoglobin and also to quantitate the possible advan-

tages arising from differences between maternal and fetal blood for gas exchange between mother and fetus.

### METHODS

Venous blood samples were obtained on thirteen pregnant women, seven of them between the fourth and eighth month of pregnancy, six of them within twelve hours before delivery. Eight blood samples were taken from the umbilical cord of infants born at term, in each case the blood being obtained within fifteen minutes of tying off the cord. Similar blood samples were obtained from the cords of two premature infants. On two of the infants born at term, repeated blood samples were taken from the jugular vein during the first month of life. Venous blood samples were also obtained from six normal non-pregnant women, since, as far as we could discover, the standard oxygen dissociation curves reported in the literature are all on men's blood.

Because of the known variability in gas content of cord blood obtained at birth, no gas analyses of the bloods as drawn were made. The blood in each case was mixed with heparin (Connaught Laboratories, Toronto), covered with heavy mineral oil to prevent evaporation, and immediately chilled in ice. All equilibrations and gas analyses were made as soon as possible, the blood being kept packed in ice except at times of equilibration. When the patient had received ether anesthesia, the ether was removed from the blood by equilibrating it with room air for fifteen minutes at 25°.

The technique of determining the oxygen dissociation curve and the CO<sub>2</sub> capacity at a pCO<sub>2</sub> of 40 mm. Hg (T<sub>50</sub>) was essentially that described in detail by Dill *et al* (14). The following changes in the procedure were made: The CO<sub>2</sub> pressure in the tonometers was set at 30 or 35 mm. Hg because it was expected that the bicarbonate content of these bloods was lower than normal. In this way we hoped to have the pH as near 7.40 as possible, to which figure the curves were finally corrected as in the original technique

$$\left( \frac{\Delta \log pO_2}{\Delta pH} = -0.479 \right).$$

The oxygen pressures were set at 5, 10, 20, 35, 50, 70 and 200 mm. Hg in order to cover a wider range than desired in the original technique. In addition, one or two points were determined in the approximate range of 50 per cent oxygen saturation at pressures of CO<sub>2</sub> of 20 and 80 mm. Hg. These points served to determine the shift in the curve with pH.

Lactic acid determinations (Edwards' modification of Friedmann, Cotonio, and Shaffer (15)) were made on all blood samples immediately before the final equilibration. The percentage of red cells in the original blood was measured by hematocrit. Serum was analyzed for sodium according to Consolazio and Dill (16), chloride according to the method outlined in Peters and Van Slyke (17), total base by the method of Consolazio and Talbott (18),

and total nitrogen by the micro Kjeldahl method (Keys' modification (19)).

The oxygen dissociation curves at pH<sub>s</sub> = 7.40 were plotted in the usual arithmetical coordinates and also on logarithmic coordinates

$$\left( \log pO_2 \text{ against } \log \frac{100 \text{ Hb}}{\text{HbO}_2} \right).$$

The latter method gives an approximate straight line, the slope of which is the constant "n" in the Hill-Barcroft equation. The axis intercept defines "K" in the same formula. These two constants were determined for each blood.

For comparison with data in the literature, and for evaluating *in vitro* conditions, the midpoint of each curve (Hb = HbO<sub>2</sub>) was also calculated at pCO<sub>2</sub> = 40, using the line charts of Henderson (20) and of Dill *et al.* (14) for the conversion.

With the value for T<sub>50</sub> of the blood as measured and the blood lactate, it was possible to determine an extrapolated T<sub>50</sub> representing the value if the lactate were 10 mgm. per cent and presenting the probable picture before the onset of labor and anesthesia. To make this calculation, use was made of the fact that lactic acid displaces bicarbonate in equimolecular amounts at the same pH. The steps in the conversion were:

- (1) Calculation of pH<sub>s</sub> at the T<sub>50</sub> point in the blood as studied.
- (2) Adding to the measured T<sub>50</sub> value an increment equivalent to the excess lactate above 10 mgm. per cent.
- (3) The pH<sub>s</sub> from (1) and the total CO<sub>2</sub> from (2) determine one point on the CO<sub>2</sub> dissociation curve of the blood in its original state; the pCO<sub>2</sub> of this point was determined from the line chart of Peters and Van Slyke (17).
- (4) The T<sub>50</sub> value of the new curve was read off from the Henderson line chart (20).

### RESULTS

The mean and total range of values of the oxygen dissociation curves between 10 and 90 per cent saturation of the three groups of bloods (non-pregnant, maternal and fetal) are presented in Figure 1, all curves being corrected to pH<sub>s</sub> 7.40. The striking feature to be observed is the position of the fetal curves: these lie to the left of both the normal and the maternal. The sole point where the curves overlap is near 90 per cent, where the technical difficulties of accurate measurement are greatest. In spite of the shift, however, inspection fails to show any striking difference in the shape of the different groups of curves.

In the same chart, comparison of the curves on the pregnant and the non-pregnant women shows that, whereas the range of the two groups



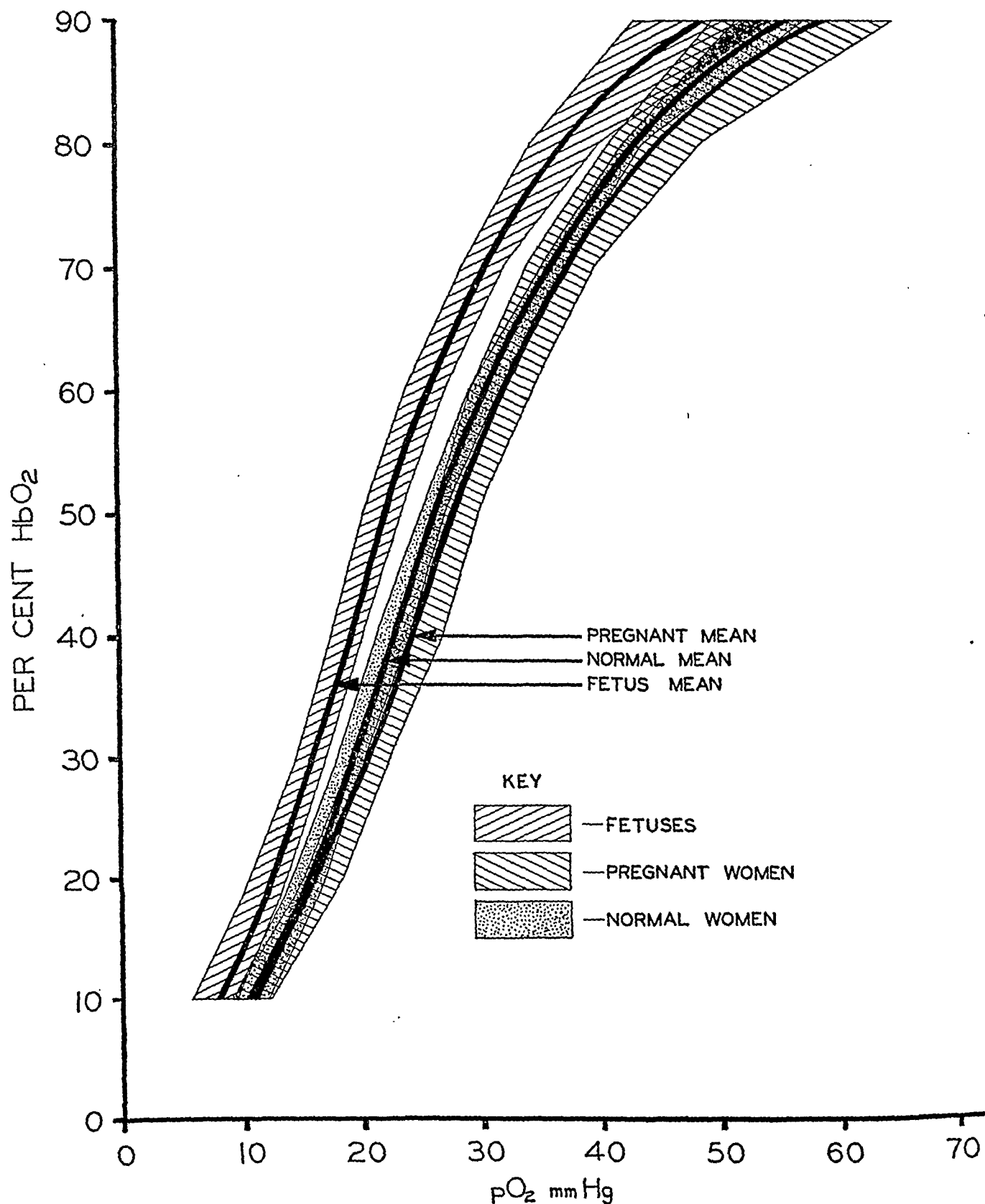


FIG. 1. MEANS AND RANGES OF OXYGEN DISSOCIATION CURVES

overlaps throughout, most of the maternal curves lie to the right and none to the left of the range of the non-pregnant women. The mean position for the dissociation curves on the blood of preg-

nant women lies slightly to the right of that of the non-pregnant.

The range and mean values of the control series of normal women shown here correspond fairly

well to the standard established from the blood of normal men (21). The mid-positions ( $\text{Hb} = \text{HbO}_2$ ) agree almost exactly (men = 26.3 mm. Hg; women = 26.0 mm. Hg). There would seem to be a slightly more sharp S-shape to the women's curve, as can be appreciated from Table I of the comparative pressures of oxygen necessary for different degrees of saturation of the hemoglobin.

TABLE I

*Comparison of oxygen dissociation curves of normal men and women at  $\text{pH}_s = 7.40$*

% HbO <sub>2</sub>	$\text{pO}_2$ (mm. Hg)	
	Normal men (Dill, Edwards and Consolazio)	Normal women (mean of 6) (This paper)
10.....	8.2	10.5
20.....	13.4	15.3
30.....	17.9	19.2
40.....	22.0	22.6
50.....	26.3	26.0
60.....	31.1	30.2
70.....	36.1	35.6
80.....	45.7	42.2
90.....	61.4	54.1

Table II presents the figures for one point on each of the curves obtained (column 2), together with other data on each blood sample. The figures in column 2 confirm the visual impression gained from the chart, namely, that fetal curves are significantly and uniformly shifted to the left and that, while the mean maternal curve lies somewhat to the right of normal, this difference is of doubtful statistical significance. The difference between the curves on the blood of pregnant and non-pregnant women appears more striking if it is noted that the range of the mid-position of the curves on the six non-pregnant women is 24.9 to 26.8 mm. Hg and that nine out of thirteen of the maternal bloods lie outside this range to the right.

The first column labelled  $T_{40}$  gives a measure of the alkali reserve in each case. It should be noted that corrections have been made whenever the lactic acid was elevated above 10 mgm. per cent, so that changes in these values are not merely temporary effects of lactic acid. The well-known progressive decrease in alkali reserve during pregnancy is again demonstrated by a comparison of the pre-term with the term groups of maternal bloods. The more marked diminution in bicar-

bonate in the fetal bloods is not so generally known and is the opposite of the findings in goat fetuses (22).

Figures in the third column are calculated values of the  $\text{pO}_2$  for half saturation of the blood at standard  $\text{pCO}_2$ . In this case the different bicarbonate values modify to some degree the relations observed at constant pH, since at constant  $\text{pCO}_2$  the bloods with the lower bicarbonate are more acid. The shift of the fetal blood curves to the left is still present but less marked; on the other hand, the maternal curves under these conditions are seen to be definitely shifted to the right.

The constants from the Hill-Barcroft equation were measured from logarithmic graphs of each curve and are presented in the fourth and fifth columns. In each case the curve approximated a straight line, the slope of which is " $n$ ." Our data indicate that " $n$ " is not significantly different in the various groups. This constancy of " $n$ " confirms our visual impression from the arithmetic curves that all were approximately the same shape. The " $K$ " values are a measure of the position of the curve; they have the same significance as oxygen pressures for half saturation. Expressed for the sake of convenience as negative logarithms, they will be seen in the fifth column to parallel the oxygen pressures at half saturation in column 2.

The values for cell volume and  $\text{HbO}_2$  capacity demonstrate anew the tendency to moderate anemia in pregnancy and the frequent polycythemia of newborn infants.

The last column shows the calculation of the factor  $\frac{\Delta \log \text{pO}_2}{\Delta \text{pH}}$ , which measures the shift in the logarithmic curve with pH. The importance of this measurement is two-fold: (1) If it differs in fetal or maternal blood from the standard established for normal man it would further establish a fundamental difference in the hemoglobin. (2) Since the oxygen dissociation curves were corrected to constant pH, using the factor for normal man, it is necessary to measure this factor in the bloods studied in order to be sure of the validity of the corrections made. Actually the factor was measured on the logarithmic curve from the equilibrium points taken at high or low  $\text{CO}_2$  pressures. While there is some scatter in the individual values for these factors, it is evi-

TABLE II

*Oxygen and carbon dioxide affinity of bloods of non-pregnant and pregnant women and of fetal blood at birth*

Non-pregnant women		$T_{40}$ corrected to normal lactate	pO <sub>2</sub> at 50 per cent oxygen saturation		Hill-Barcroft formula constants		Cell volume	HbO <sub>2</sub> capacity	$\Delta \log pO_2$ $\Delta pH$
			pH <sub>s</sub> = 7.40	pCO <sub>2</sub> = 40	$n$	$-\log K$			
		<i>volume per cent</i>	<i>mm. Hg</i>				<i>per cent</i>	<i>volume per cent</i>	
Ca		48.7	26.4	25.5	2.72	3.86	42.3	18.02	
Ja		49.9	26.8	25.9	2.61	3.72		17.02	
Te		42.7	24.9	25.2	2.61	3.64	43.7	19.61	
Co		48.5	26.5	25.8	2.78	3.96	38.1	16.47	
Na		45.8	25.8	26.2	2.63	3.71	45.4	17.37	
Se		44.6	25.8	26.6	2.53	3.57	40.3	16.82	
Mean		46.70	26.03	25.87	2.647	3.743	41.96	17.55	
$\sigma M$		1.14	0.28	0.21	0.037	0.059	1.28	0.465	
Pregnant women									
Pre-term	Month of pregnancy								
Ku	4	43.8	25.8	26.6	2.78	3.92	38.5	16.63	-0.53
Br	5	43.7	29.4	30.8	2.50	3.67	33.8	15.00	-0.48
Co	5	45.4	28.2	28.5	2.68	3.87	31.4	15.78	-0.45
O'B	5½	43.5	25.9	26.9	2.76	3.89	33.9	14.25	-0.47
Gr	7½	42.6	27.6	29.0	2.52	3.64	37.2	16.13	-0.38
Co	8	45.0	28.7	29.5	2.52	3.67	31.1	13.67	-0.50
Ma	8½	47.7	29.5	29.2	2.72	4.00	37.9	16.06	
Mean		44.53	27.87	28.64	2.640	3.809	34.83	15.36	-0.469
$\sigma M$		0.64	0.58	0.56	0.046	0.055	1.16	0.411	0.020
Term									
Ba		41.4	26.1	27.9	2.59	3.67	37.4	15.98	-0.50
Fl		40.3	26.5	29.1	2.50	3.56	36.8	16.70	-0.45
Hi		36.0	27.0	30.1	2.79	3.99	30.4	15.50	
Po		41.8	27.0	28.5	2.50	3.58	41.1	16.97	-0.49
McG		47.0	27.8	27.8	2.48	3.58	36.3	15.41	-0.47
An		39.7	29.2	31.1	2.72	3.97	35.9	17.40	-0.56
Mean		41.03	27.27	29.08	2.597	3.725	36.32	16.33	-0.494
$\sigma M$		1.46	0.45	0.53	0.053	0.082	1.41	0.334	0.016
Mean of all pregnant		42.92	27.59	28.85	2.620	3.770	35.52	15.81	-0.480
$\sigma M$		0.88	0.37	0.38	0.034	0.047	0.88	0.294	0.014
Fetus									
Term									
Fl		35.9	22.3	24.3	2.51	3.38		22.68	
Hi		38.0	21.6	23.2	2.35	3.13	51.1	19.81	-0.40
Po		35.6	20.9	22.8	2.54	3.35	50.6	22.46	-0.52
McG		34.5	23.2	25.6	2.49	3.40	57.6	24.53	-0.52
An		31.1	22.0	25.4	2.60	3.49	59.6	25.40	-0.49
Ro		33.4	20.0	22.3	2.50	3.25	60.0	24.36	-0.52
Wh		33.5	21.4	23.7	2.72	3.62	59.2	25.61	
Bo		37.6	21.9	24.2	2.59	3.47	44.5	18.15	
Premature									
Ma (7th month)		40.4	22.4	23.4	2.70	3.64	43.1	20.46	-0.57
Pa (5th month)		30.9	22.9	27.3	2.69	3.65	49.9	20.63	-0.47
Mean		35.09	21.86	23.94	2.569	3.438	52.84	22.41	-0.499
$\sigma M$		0.96	0.30	0.42	0.036	0.054	2.18	0.813	0.020

dent that there is no significant deviation in the mean values either between the different groups or between any of them and the value of  $-0.48$  which has been found for the blood of normal men (14).

The data on the two premature infants are remarkable only for the lack of differentiation from the term infants. The oxygen dissociation curves are well within the range observed on infants at term. As expected, neither of them showed polycythemia, which from available data seems to be a development of the last two months of fetal life (23).

Table III presents the results of some analyses on the sera of these bloods. The bicarbonate values at the arbitrary  $p\text{CO}_2$  of 40 are calculated from the  $\text{CO}_2$  content of the whole blood equilibrated at a known  $p\text{CO}_2$  according to Peters and Van Slyke. It is obvious that no anion-cation balance sheet can be made since the bicarbonate

TABLE III

*Analyses of serum of pregnant women and fetuses at birth*

Pregnant women		Sodium	Chloride	Total base	$\text{HCO}_3^-$ at $p\text{CO}_2=40$	Total nitrogen
Pre-term	Month of pregnancy					
		m. Eq. per liter	m. Eq. per liter	m. Eq. per liter	m. Eq. per liter	grams per liter
Ku	4	138.2	106.3		22.7	11.18
Br	5	136.1	108.7		22.5	10.41
Co	5	135.6	107.2		23.3	11.06
O'B	5½	135.6	106.9		22.0	11.30
Gr	7½		100.1		22.1	11.41
Co	8	135.1	103.6		22.6	10.53
Ma	8½	140.0	103.3		24.8	
Term						
Ba			105.2		21.4	10.43
Fl		136.1	110.3		22.4	11.02
Hi			103.7		18.3	10.90
Po			106.7		21.9	10.72
McG		140.9	108.2	154.2	24.2	9.98
An		140.2	109.5		20.8	10.06
Fetus Term						
Fl			111.3		20.4	11.34
Hi		139.8	108.1		20.6	
Po		130.9	104.2		20.0	10.32
McG		129.1	107.4	157.0	19.9	9.47
An			104.1		18.2	10.58
Ro		127.3	103.9		19.3	11.17
Wh		136.8	110.9		19.7	9.26
Bo		136.1	112.9	154.3	19.9	7.55
Premature						
Ma (7th month)		126.8	109.5		22.2	8.44
Pa (5th month)		125.1	113.6	135.7	16.9	6.07

TABLE IV

*Changes in blood during the first month of life*

Subject	Age	$T_{40}$ corrected to normal lactate	$p\text{O}_2$ at 50 per cent saturation		Cell volume	$\text{HbO}_2$ capacity
			$p\text{H}_s=7.40$	$p\text{CO}_2=40$		
		volume per cent	mm. Hg		per cent	volume per cent
Wh	Birth	33.5	21.4	23.7	59.2	25.61
	1 day	42.0	22.2	22.7	49.3	21.85
	5 days	42.2	23.3	23.8	43.1	20.21
	34 days	41.7	27.7	29.2		17.90
Bo	Birth	37.6	21.9	24.2	44.5	18.15
	3 days	39.0	23.0	24.1	53.4	23.15
	34 days	49.7	25.1	24.3	35.5	14.83

values are taken at arbitrary conditions and not those *in vivo*. From the point of view of the possible effect of salt concentration on the oxygen dissociation curve, it can be concluded, however, that changes in the serum are too small to explain the shift in the fetal oxygen dissociation curve. The changes in some of the separate ionic concentrations, however, are of interest. A tendency to a high chloride content, especially in the fetal bloods, is associated with a low bicarbonate concentration.

The most striking abnormalities are seen in the two bloods of prematurely born infants. In both, the serum sodium is definitely low; this finding is shared by two of the term infants but by no others. Even more remarkably low is the total base value for one of the premature infants. However, this value is normal for two term infants and one pregnant woman on whom the determination was carried out. Likewise, in the two premature infants' blood, the serum nitrogen (a measure of serum protein) is definitely lowered. The significance of these chemical changes is not known and their finding may be considered incidental to the present work. It is of interest to speculate, however, on whether such chemical deficiencies may not be significant physiological handicaps to premature infants.

Table IV presents the data on two infants whose blood was tested throughout the first month of life. It will be seen from the progressive increase in the figures of the midpoints of the oxygen dissociation curves that the curves at constant  $p\text{H}_s$  shifted gradually to the right so that they were within the normal range at thirty days

TABLE II

*Oxygen and carbon dioxide affinity of bloods of non-pregnant and pregnant women and of fetal blood at birth*

Non-pregnant women		$T_{40}$ corrected to normal lactate	pO <sub>2</sub> at 50 per cent oxygen saturation		Hill-Barcroft formula constants		Cell volume	HbO <sub>2</sub> capacity	$\Delta \log pO_2$ $\Delta pH$
			pH <sub>s</sub> = 7.40	pCO <sub>2</sub> = 40	"	-log K			
		volume per cent	mm. Hg				per cent	volume per cent	
Ca		48.7	26.4	25.5	2.72	3.86	42.3	18.02	
Ja		49.9	26.8	25.9	2.61	3.72		17.02	
Te		42.7	24.9	25.2	2.61	3.64	43.7	19.61	
Co		48.5	26.5	25.8	2.78	3.96	38.1	16.47	
Na		45.8	25.8	26.2	2.63	3.71	45.4	17.37	
Se		44.6	25.8	26.6	2.53	3.57	40.3	16.82	
Mean		46.70	26.03	25.87	2.647	3.743	41.96	17.55	
$\sigma M$		1.14	0.28	0.21	0.037	0.059	1.28	0.465	
Pregnant women									
Pre-term	Month of pregnancy								
Ku	4	43.8	25.8	26.6	2.78	3.92	38.5	16.63	-0.53
Br	5	43.7	29.4	30.8	2.50	3.67	33.8	15.00	-0.48
Co	5	45.4	28.2	28.5	2.68	3.87	31.4	15.78	-0.45
O'B	5½	43.5	25.9	26.9	2.76	3.89	33.9	14.25	-0.47
Gr	7½	42.6	27.6	29.0	2.52	3.64	37.2	16.13	-0.38
Co	8	45.0	28.7	29.5	2.52	3.67	31.1	13.67	-0.50
Ma	8½	47.7	29.5	29.2	2.72	4.00	37.9	16.06	
Mean		44.53	27.87	28.64	2.640	3.809	34.83	15.36	-0.469
$\sigma M$		0.64	0.58	0.56	0.046	0.055	1.16	0.411	0.020
Term									
Ba		41.4	26.1	27.9	2.59	3.67	37.4	15.98	-0.50
Fl		40.3	26.5	29.1	2.50	3.56	36.8	16.70	-0.45
Hi		36.0	27.0	30.1	2.79	3.99	30.4	15.50	
Po		41.8	27.0	28.5	2.50	3.58	41.1	16.97	-0.49
McG		47.0	27.8	27.8	2.48	3.58	36.3	15.41	-0.47
An		39.7	29.2	31.1	2.72	3.97	35.9	17.40	-0.56
Mean		41.03	27.27	29.08	2.597	3.725	36.32	16.33	-0.494
$\sigma M$		1.46	0.45	0.53	0.053	0.082	1.41	0.334	0.016
Mean of all pregnant		42.92	27.59	28.85	2.620	3.770	35.52	15.81	-0.480
$\sigma M$		0.88	0.37	0.38	0.034	0.047	0.88	0.294	0.014
Fetus									
Term									
Fl		35.9	22.3	24.3	2.51	3.38		22.68	
Hi		38.0	21.6	23.2	2.35	3.13	51.1	19.81	-0.40
Po		35.6	20.9	22.8	2.54	3.35	50.6	22.46	-0.52
McG		34.5	23.2	25.6	2.49	3.40	57.6	24.53	-0.52
An		31.1	22.0	25.4	2.60	3.49	59.6	25.40	-0.49
Ro		33.4	20.0	22.3	2.50	3.25	60.0	24.36	-0.52
Wh		33.5	21.4	23.7	2.72	3.62	59.2	25.61	
Bo		37.6	21.9	24.2	2.59	3.47	44.5	18.15	
Premature									
Ma (7th month)		40.4	22.4	23.4	2.70	3.64	43.1	20.46	-0.57
Pa (5th month)		30.9	22.9	27.3	2.69	3.65	49.9	20.63	-0.47
Mean		35.09	21.86	23.94	2.569	3.438	52.84	22.41	-0.499
$\sigma M$		0.96	0.30	0.42	0.036	0.054	2.18	0.813	0.020

dent that there is no significant deviation in the mean values either between the different groups or between any of them and the value of  $-0.48$  which has been found for the blood of normal men (14).

The data on the two premature infants are remarkable only for the lack of differentiation from the term infants. The oxygen dissociation curves are well within the range observed on infants at term. As expected, neither of them showed polycythemia, which from available data seems to be a development of the last two months of fetal life (23).

Table III presents the results of some analyses on the sera of these bloods. The bicarbonate values at the arbitrary  $pCO_2$  of 40 are calculated from the  $CO_2$  content of the whole blood equilibrated at a known  $pCO_2$  according to Peters and Van Slyke. It is obvious that no anion-cation balance sheet can be made since the bicarbonate

TABLE III

*Analyses of serum of pregnant women and fetuses at birth*

Pregnant women		Sodium	Chloride	Total base	$HCO_3^-$ at $pCO_2 = 40$	Total nitrogen
Pre-term	Month of pregnancy					
		<i>m. Eq. per liter</i>	<i>m. Eq. per liter</i>	<i>m. Eq. per liter</i>	<i>m. Eq. per liter</i>	<i>grams per liter</i>
Ku	4	138.2	106.3		22.7	11.18
Br	5	136.1	108.7		22.5	10.41
Co	5	135.6	107.2		23.3	11.06
O'B	5½	135.6	106.9		22.0	11.30
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TABLE IV

*Changes in blood during the first month of life*

Subject	Age	$T_{50}$ corrected to normal lactate	$pO_2$ at 50 per cent saturation		Cell volume	$HbO_2$ capacity
			$pH_s = 7.40$	$pCO_2 = 40$		
		<i>volume per cent</i>	<i>mm. Hg</i>		<i>per cent</i>	<i>volume per cent</i>
Wh	Birth	33.5	21.4	23.7	59.2	25.61
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	5 days	42.2	23.3	23.8	43.1	20.21
	34 days	41.7	22.7	29.2		17.90
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values are taken at arbitrary conditions and not those *in vivo*. From the point of view of the possible effect of salt concentration on the oxygen dissociation curve, it can be concluded, however, that changes in the serum are too small to explain the shift in the fetal oxygen dissociation curve. The changes in some of the separate ionic concentrations, however, are of interest. A tendency to a high chloride content, especially in the fetal bloods, is associated with a low bicarbonate concentration.

The most striking abnormalities are seen in the two bloods of prematurely born infants. In both, the serum sodium is definitely low; this finding is shared by two of the term infants but by no others. Even more remarkably low is the total base value for one of the premature infants. However, this value is normal for two term infants and one pregnant woman on whom the determination was carried out. Likewise, in the two premature infants' blood, the serum nitrogen (a measure of serum protein) is definitely lowered. The significance of these chemical changes is not known and their finding may be considered incidental to the present work. It is of interest to speculate, however, on whether such chemical deficiencies may not be significant physiological handicaps to premature infants.

Table IV presents the data on two infants whose blood was tested throughout the first month of life. It will be seen from the progressive increase in the figures of the midpoints of the oxygen dissociation curves that the curves at constant  $pH_s$  shifted gradually to the right so that they were within the normal range at thirty days

of age. Concomitantly, there was a rise in bicarbonate and a fall in  $\text{HbO}_2$  capacity. The effect of the former is to make the change in the oxygen dissociation curve at constant  $\text{pCO}_2$  less striking than at constant  $\text{pH}$ , as the third column in the table indicates. This is due to the fact that, as measured at constant  $\text{pCO}_2$ , the blood becomes increasingly alkaline as the bicarbonate rises.

#### DISCUSSION

The significance of the observed characteristics of fetal, maternal, and normal blood may be discussed profitably from two points of view. In the first place, there is the theoretical question of a chemically different hemoglobin in the fetus. The second point of view is the physiological, according to which we may inquire into the advantages or disadvantages to the fetus of the different properties which its blood displays.

The data presented show that the fetal blood has a greater affinity for oxygen than the blood of pregnant and non-pregnant women over the entire range of oxygen pressures. These comparisons were made at the same  $\text{pH}$  of the serum. In order to be sure that the hemoglobins are inherently different, it would be necessary to compare them at the same environmental  $\text{pH}$ , namely that of the cell. Such values were not measured directly but they may be calculated from the values for the serum constituents, assuming normal permeability of the cell membrane. Thus from our data we may conclude that *if the cell membrane of the fetal red cell has the same characteristics of permeability as that of the adult cell, then the fetal hemoglobin has truly different properties*. Unfortunately we have no positive evidence by which we make this assumption of constant permeability. In fact, there is the evidence of Andreen-Svedberg (24) that calves' corpuscles are more permeable to glucose than cows' corpuscles, which gives a hint that cell membranes of young animals may differ from those of adults.

Thus the evidence for a chemically different human fetal hemoglobin is not clinched. That for goats is much surer, since the same differences were observed on buffered hemoglobin solutions at the same  $\text{pH}$  and also because the fetal curve was invariably found to have a different shape from the adult. Our evidence fails to confirm a

characteristic fetal shape to the curve in humans, so one link in the evidence for a true human fetal hemoglobin is lacking. The final answer will await more knowledge of fetal red cell permeability and a further study of human hemoglobin solutions.

From the point of view of applied physiology the preceding considerations are less important. There can be no doubt that under the same serum conditions of  $\text{pH}$  the fetal blood will take up more oxygen in the placenta than would adult human blood. By the same token it will be able to give up less of its oxygen at the same oxygen tension in the fetal tissues. Since, however, it is established that fetal tissues use less oxygen per gram of weight than adult tissues (25), presumably due to the fact that heat loss is minimal *in utero*, the latter physiological disadvantage may not be important.

Granted that there seems to be a physiological advantage to the fetus provided the serum  $\text{pH}$  of mother and fetus is identical, we must next inquire as to how much this concept is actually modified by possible differences in the  $\text{pH}$  of the two bloods. The lowered bicarbonate in both mother and fetus tends to make the serum more acid than normal unless compensatory adjustments are made. In the case of the mother such compensatory regulation is brought about by the respiratory center, so that *in vivo* her blood approaches conditions of standard  $\text{pH}$ . The fetus, on the other hand, has no central respiratory control of acid-base balance; the  $\text{pH}$  of its serum is determined by a balance between the  $\text{CO}_2$  removal through the placenta and the  $\text{CO}_2$  given up by the tissues. Thus our figures for the fetal blood at constant  $\text{pCO}_2$  approach more nearly those *in vivo* than do the figures at constant  $\text{pH}$ . As may be noted from Table II, a comparison of such figures (mother at  $\text{pH}$  7.40, fetus at  $\text{pCO}_2$  40) brings the fetal and maternal curves closer together. Unfortunately, accurate figures of the  $\text{pCO}_2$  of the fetal blood in *utero* are not available. It is quite possible that the fetal  $\text{pCO}_2$  *in vivo* is appreciably higher than 40 mm. Hg; in which case the fetal and maternal curves will be even closer together. Thus there are two opposing forces acting in the case of the fetal blood: an inherent greater affinity for oxygen facilitating the taking up of oxygen and a physiologically more acid state

opposing it. It is likely, however, that the former force is somewhat greater, so that the fetal blood operates at an advantage in becoming promptly saturated with oxygen in its passage through the placenta.

#### SUMMARY AND CONCLUSIONS

1. Blood was obtained from thirteen pregnant women, six non-pregnant women and eight human fetuses at term birth, and the oxygen dissociation curves, together with certain serum electrolytes, were determined.

2. The oxygen dissociation curve of fetal bloods at constant pH<sub>s</sub> is displaced to the left compared with that of pregnant and non-pregnant women. This suggests but does not prove the existence of qualitatively distinctive fetal hemoglobin.

3. The mean oxygen dissociation curve of maternal blood shows only a doubtful deviation from that of the non-pregnant women, although more than one-half of the individual curves were displaced to the right.

4. The oxygen dissociation curves of two premature infants were not different from those of infants at term.

5. The oxygen dissociation curve after birth shifts to the right so that within thirty days it is like that of the normal adult.

6. The alkali reserve of fetal blood is markedly lowered; that of pregnant women moderately so. This difference makes it probable that the difference in oxygen curves is less marked *in vivo* than at constant pH<sub>s</sub>.

7. The sera of the two premature infants, as well as those of two of the infants born at term, showed low serum sodium values.

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# METABOLIC STUDIES IN PATIENTS WITH CANCER OF THE GASTRO-INTESTINAL TRACT. I. PLASMA VITAMIN A LEVELS IN PATIENTS WITH MALIGNANT NEOPLASTIC DISEASE, PARTICULARLY OF THE GASTRO-INTESTINAL TRACT<sup>1</sup>

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Cancer of the gastro-intestinal tract accounts for 54 per cent of the total mortality from neoplastic disease in males (1)—an incidence which warrants particularly intensive study of the disorder. The associated metabolic abnormalities are especially important, not only because through them some clue as to etiology may be found, but also to provide information by which the mortality from difficult operative or radiological procedures may be reduced.

The results of numerous clinical and experimental studies suggest that dietary factors are important in the maintenance of a normal gastro-intestinal mucous membrane (2, 3). Hence, close attention has been given, in the Memorial Hospital, to the study of the nutritional status of patients with intestinal neoplasms. The results of the measurement of levels of vitamin A in the plasma, herein reported, are considered to be of special interest.

## MATERIAL

Normal individuals were studied to establish the normal levels of vitamin A, carotene and thiamin in the blood, as well as of riboflavin in the urine. Adult laboratory technicians, house physicians, nurses, and normal young men, all apparently in good health and on adequate diets, were used as subjects.

The clinical material upon which this report is based includes six different groups of patients. The first is composed of 51 patients with cancer of the gastro-intestinal tract, selected from 62 consecutive admissions to the gastric and rectal services of the Memorial Hospital. Eleven of the 62 cases were discarded because they were febrile or had received medication which might provide misleading findings.

<sup>1</sup>The author gratefully acknowledges the assistance given by the Jane Coffin Childs Fund, the Standard Brands, Inc., and the Dazian Foundation.

<sup>2</sup>Finney-Howell Fellow.

The diagnosis of gastro-intestinal neoplasm was made by x-ray, endoscopic procedure, laparotomy, biopsy, or a combination of these methods. Of the 51 patients studied, 28 had gastric, 18 rectal, and 5 esophageal cancer. Thirty-seven of the patients provided an opportunity for examination of the liver, either at autopsy or at operation. In 11, metastatic nodules were found, but in none were they sufficiently large to suggest the possibility of anatomical impairment of hepatic function.

Whereas all but 4 of the patients eventually exhibited the symptoms commonly associated with their disease—principally weight loss, anorexia, changes in bowel habits, and abdominal pain or tenderness—only 6 had had nausea, vomiting, diarrhea or melena. All had various degrees of anemia. Fifteen had palpable abdominal or rectal masses.

Thirteen of the 51 patients had been on restricted diets, but the restriction did not apply to the intake of vitamin-containing materials. Indeed, most of the patients had taken a diet which was abnormally high in its content of carotenoids.

The second group of cases comprises 14 patients with benign gastro-intestinal lesions. Eight had peptic ulcers associated with continuous vomiting, anorexia, and weight loss. Of the remaining 6, 2 had severe ulcerative colitis, 2 long-standing active sprue, and 2 mucous colitis; all 6 had had persistent diarrhea for from 1 to 10 years.

The third group consists of 19 patients whose gastro-intestinal neoplasm had been removed surgically. In 12, the lesion originally was of the stomach, and in 7, of the large bowel. At the time of laparotomy hepatic metastases had been present in only 1 of the 19, and that patient is now alive 5 years after the operation. When the vitamin A levels were determined in the plasma of the 19 post-operative patients, all were free of symptoms. Three of the 19 had been on grossly deficient diets for at least 4 months.

The fourth group of patients represents 20 routine admissions to the Head and Neck Service of the Hospital. These all had leukoplakia of the oral mucosa, without cancer or syphilis, and 5 had been on grossly deficient diets.

The fifth group consists of 13 patients admitted to the Gastric Service. In all, atrophy of the gastric mucosa was found by gastroscopic examination, all had symptoms

referable to their disease, and 4 had been on grossly deficient diets.

In addition to the patients discussed, the vitamin A levels were ascertained in the plasma of several smaller groups of individuals with cancer located elsewhere than in the gastro-intestinal tract. These represent routine consecutive admissions of 69 patients with lymphomas, 8 with osteogenic sarcoma, 8 with cancer of the pancreas, 20 with cancer of the breast, and 6 with cancer of the mouth.

## METHODS

Vitamin assays were not made on any patient until an adequate hospital diet had been taken for at least 2 days, and in the patients who had undergone operation no tests were made during the first 4 post-operative days. All the venipunctures were made in the morning when the patients were fasting. In most instances, the vitamin A and carotene levels were checked by repeated determinations.

1. *Vitamin A and carotene in plasma.* The method employed for the measurement of vitamin A was the Carr-Price reaction, adapted for the photoelectric colorimeter by Dann and Evelyn (4) and modified for blood determinations by Clausen and McCoord (5) and by Kimble (6). It has been further modified for use as herein described.

The instrument used was the Pfaltz and Bauer fluorophotometer. Filter combinations Number 629 with peak at 6200 Å and Number 635 with peak at 4400 Å were used for vitamin A and carotene, respectively.

The standard curves for carotene were obtained from measurements of various dilutions of pure beta-carotene. Two different samples of carotene were employed, one manufactured by the S. M. A. Corporation and the other by the Hoffman-LaRoche Company (Figure 1).

For the vitamin A standard, the U.S.P. Reference Cod Liver Oil Number 2 was used, as well as other sources. These were first saponified and then treated as described by Koehn and Sherman (7). For the final standardization, a vitamin A concentrate, E (1 per cent, 1 cm.) 107

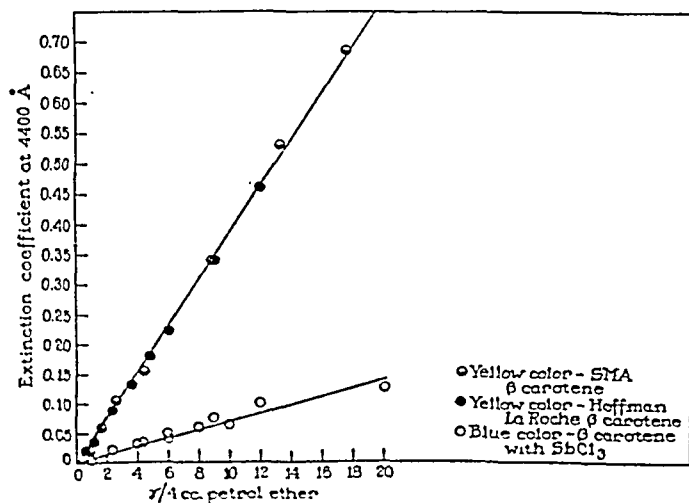


FIG. 1. STANDARD CURVE FOR CAROTENE

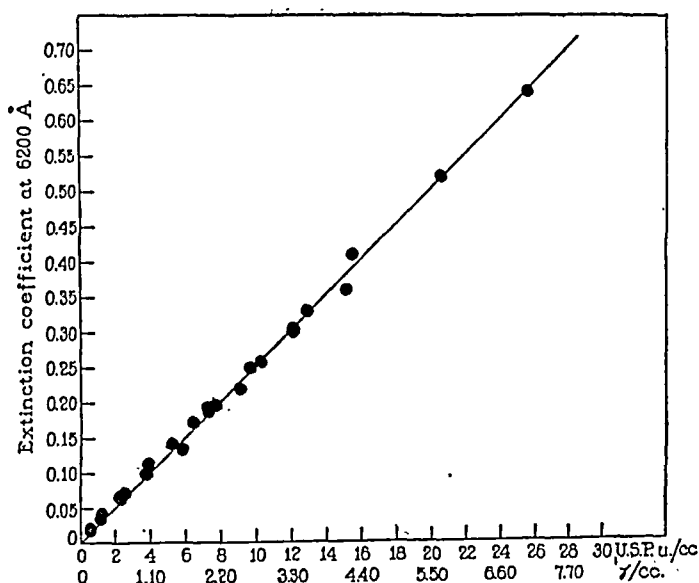


FIG. 2. VITAMIN A STANDARD CURVE—VITAMIN A CONCENTRATE E (1 PER CENT 1 CM., 107) 328  $m\mu$  (DISTILLATION PRODUCTS, INC.)

at 328  $m\mu$ , supplied by the Distillation Products, Inc., was employed. Because of its high potency (224,700 U.S.P. units per gram of oil) this oil was not saponified but was taken up directly in chloroform, in which solvent the vitamin A measurements were made. The data reported here are based on the curve computed from this standard oil (Figure 2).

Five cc. of the plasma, 5 cc. of 95 per cent alcohol, and 5 cc. of petrol ether were shaken in a stoppered centrifuge tube for 10 minutes. The layers were separated by centrifugation, and 4 cc. of the petrol ether layer were pipetted into a 10 cc. colorimeter tube 12 mm. in diameter. The tube was stoppered and the yellow color of the carotene was read. The petrol ether was evaporated off under a stream of nitrogen and the residue taken up in 1 cc. of chloroform (reagent quality). With the tube in the instrument, 3.5 cc. of antimony trichloride reagent (225 grams  $SbCl_3$  per liter of washed chloroform) were added from a rapidly draining pipette and the maximum extinction coefficient of the blue color was measured. The extinction coefficient value was converted into U.S.P. units of vitamin A by reference to the standard calibration curves (Figures 1 and 2).

2. *Thiamin in white cells.* The thiamin measurements in our investigation were made by the technique developed by Atkin, Schultz and Frey (8). This method is based on the principle that the fermentation by yeast of a glucose-salt mixture to carbon dioxide is accelerated within certain limits in direct proportion to the concentration of thiamin present. This reaction is measured in a Warburg vessel under anaerobic conditions.

To obtain leukocytes, whole oxalated blood was allowed to settle and the cloudy supernatant plasma was removed and centrifuged at 3,000 r.p.m. The clear plasma was poured off and the clump of leukocytes in the bottom of the tube was taken up in a small amount of saline, mixed and transferred by a capillary pipette to a graduated

hematocrit tube. After the hematocrit reading was obtained and the actual volume of cells calculated, the cells were diluted with isotonic saline to the optimum concentration for determinations of their thiamin content.

3. *Riboflavin in the urine* was determined by the method of Ferrebee (9).

## RESULTS

The results of the experiments herein described group themselves naturally under three headings: (A) The levels of vitamin A and carotene in the plasma of normal individuals and of patients with gastro-intestinal cancer and other disorders. (B) Evidence for the cause of the abnormalities discovered in these patients. (C) The effects of the administration of substances containing other vitamins on the plasma levels of vitamin A in the various groups of patients.

### A. Vitamin A and carotene levels in the plasma of normal individuals and of patients with gastro-intestinal cancer

The mean average<sup>a</sup> plasma vitamin A of 62 normal males who were studied as controls was 170.3 U.S.P. units per 100 cc.; the standard deviation ( $\sigma_1$ ) was  $\pm 38$ , and the normal range,



FIG. 3. PLASMA VITAMIN A AND CAROTENE LEVELS OF NORMAL MALES

therefore, from 132 to 208 U.S.P. units. The mean average carotene of this group was 0.21 mgm. per 100 cc.; the standard deviation ( $\sigma_1$ ) was  $\pm 0.13$  and the normal range from 0.09 to 0.34 mgm. per cent (Figure 3). A similar study of

62 normal females gave a mean average plasma vitamin A level of 149.1 U.S.P. units per 100 cc., and a standard deviation ( $\sigma_1$ ) of  $\pm 46$ . The normal range was from 103.1 to 195.1 U.S.P. units. The mean average carotene level of the female group was 0.18 mgm. per 100 cc., the standard deviation ( $\sigma_1$ )  $\pm 0.10$ , and normal range from 0.08 to 0.40 mgm. per cent (Figure 4). As

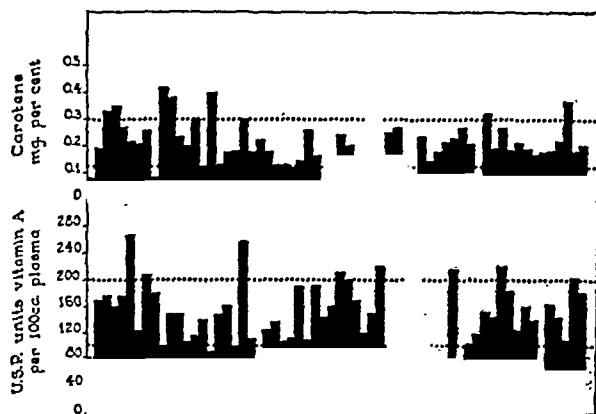


FIG. 4. PLASMA VITAMIN A AND CAROTENE LEVELS OF NORMAL FEMALES

long as the individuals in this study, both normal and those with cancer, remained untreated and on the same diet, their plasma levels of vitamin A never varied more than  $\pm 10$  U.S.P. units from day to day.

The findings in the plasma of 38 males with cancer of the gastro-intestinal tract were in sharp contrast to those in normal males. The vitamin A levels ranged from 32 to 180 U.S.P. units per 100 cc. (Figure 5), and averaged 84 U.S.P. units, or about one-half the normal value. Of the 38 male patients, 34 had levels below the normal range. The average carotene of the 38 males was 0.14 mgm. per 100 cc., and ranged from 0.04 to 0.50 mgm. per 100 cc. Study of the 13 female patients in the group provided similar findings. The plasma vitamin A levels ranged from 50 to 148 U.S.P. units per 100 cc. (Figure 6), and the average was 78.5 U.S.P. units—again about half the normal value. Eleven of the 13 had levels below the normal range. The average carotene in the plasma of the 13 females was 0.135 mgm. per 100 cc., and the levels ranged from 0.05 to 0.27 mgm. per 100 cc.

<sup>a</sup> Statistical analysis was not applied to the values of vitamin A and carotene in the plasmas of the various groups of patients studied because several of those groups consisted of comparatively small numbers of individuals.

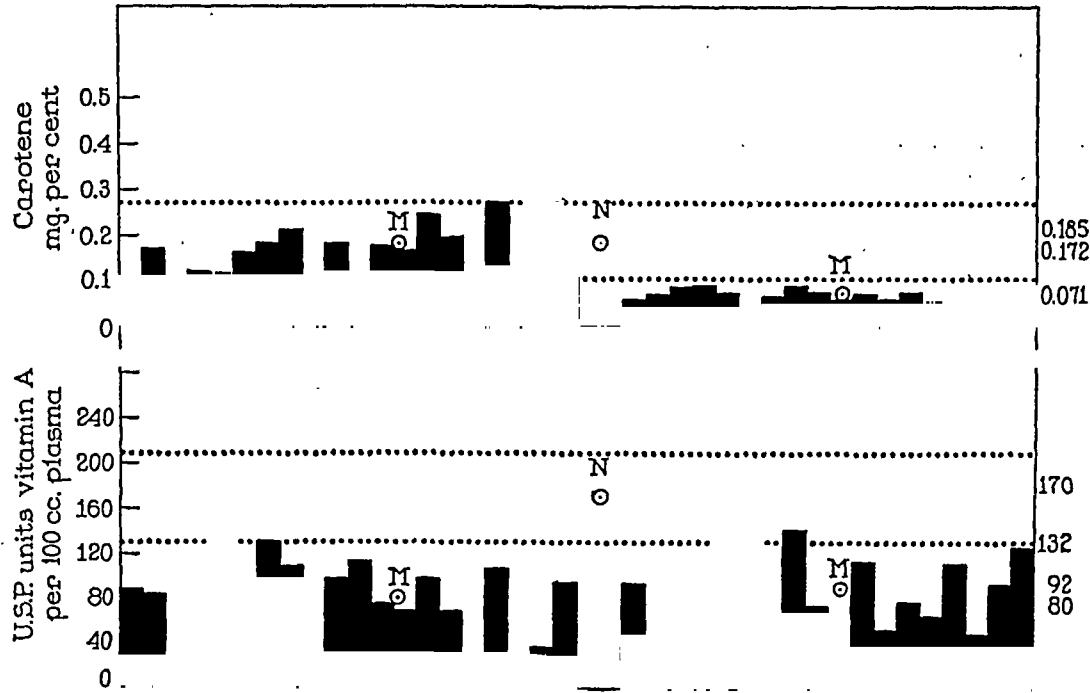


FIG. 5. PLASMA VITAMIN A AND CAROTENE LEVELS OF MALES WITH CANCER OF THE GASTRO-INTESTINAL TRACT

Of all the 51 patients, 86 per cent had vitamin A levels below the normal range, and 96 per cent had levels lower than the average.

*B. Experiments to determine the cause of the low plasma vitamin A levels in plasma of patients with gastro-intestinal cancer*

The most obvious explanation of the findings of persistently low levels of vitamin A in the blood was that, since the patients had gastro-intestinal disease, they were malnourished. If this were the case, one would expect to find a deficiency of other vitamins as well as of vitamin A in the plasma of these patients. Since carotene is a precursor of vitamin A, it seemed important to investigate the plasma levels of carotene, particularly in those patients who were already known to have taken restricted diets.

The dietary history of the 51 patients studied revealed that only 13 had been on diets deficient in calories, vitamins, or both. Of these 13 patients, 4 had cancer of the esophagus, 8 cancer of the stomach, and 1 cancer of the colon. Eleven were found to have plasma levels of vitamin A below the normal limits, but 4 of the 11 had plasma carotene levels within the normal range. Normal carotene levels also were found in the plasma of the 2 patients who had been on deficient diets but had normal plasma levels of vitamin A.

Of the 51 patients, 23 showed low plasma levels of carotene as well as of vitamin A. Included in this group were 7 of the 13 patients who had been on restricted diets, as described in the preceding paragraph. No further studies were made of these 23 patients, since it seemed quite possible that their dietary intake of carotenoids had been

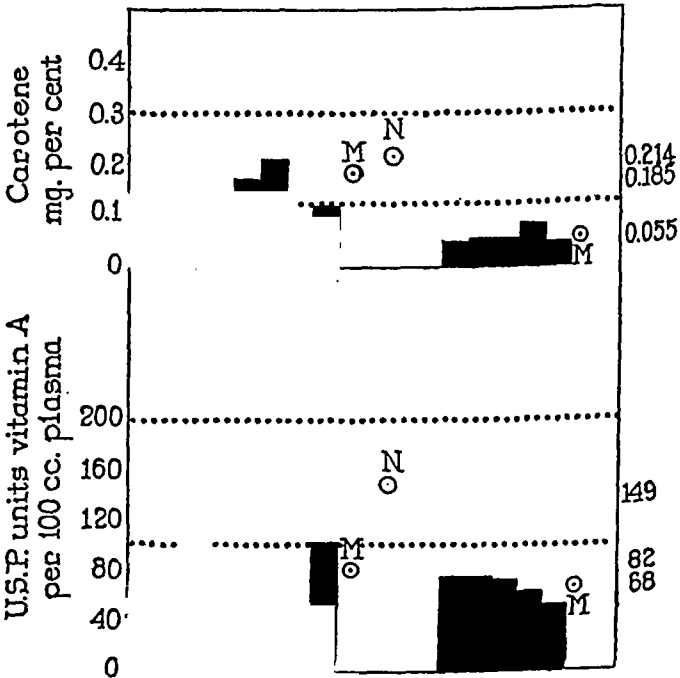


FIG. 6. PLASMA VITAMIN A AND CAROTENE LEVELS OF FEMALES WITH CANCER OF THE GASTRO-INTESTINAL TRACT

insufficient and so a simple dietary vitamin A deficiency had resulted.

The remainder of the patients, however, 28 in number, showed normal or actually elevated levels of carotene. All but 5 of them had vitamin A levels below the normal range, and all were considerably below the average. It would appear, then, that these 28 patients had neither a deficiency of carotenoids in the diet, nor malabsorption of carotenoids from the gastro-intestinal tract.

If carotene levels of the plasma are an index as to the availability of carotene for conversion to Vitamin A, then the low levels of the vitamin in these 28 patients could not be due to dietary deficiency. Bessey and Wolbach (10), however, have concluded from their studies that there is no association between the amount of carotene ingested and its level in the plasma. Hence, it was possible that the individuals with normal carotene levels who made up the second group really had ingested an insufficient amount of vitamin A. If this were true, and their dietary intake had been inadequate, it might well be reflected in a deficiency of other dietary constituents as well, and it was necessary to investigate this possibility before conclusions could be drawn as to the dietary status of the patients.

Accordingly, in 18 of the 28 patients the thiamin concentration in the leukocytes and the excretion of riboflavin in the urine were measured (Figure 7). These determinations could not be made on the remaining 10 patients of this group. To ascertain the normal values, the leukocyte thiamin of 18 normal men and women was measured, as was the riboflavin excretion in the urine of 20 normal individuals of both sexes. The average leukocyte thiamin was 90 gamma per 100 cc., and the range from 50 to 150 gamma per cent. No sex difference was observed. The amount of riboflavin excreted in the urine varied from 200 to 900 gamma per day. These values agree well with those reported by Axelrod *et al.* (11).

The thiamin concentration in the leukocytes of the 18 patients ranged from 52 gamma to 244 gamma per 100 cc., all within the normal limits. The average was 148 gamma per 100 cc. of leukocytes, a figure significantly higher than normal (90 gamma per 100 cc.). Why this group should

have a high concentration of thiamin in their leukocytes associated with low plasma levels of vitamin A is unknown.

Similarly, the urinary excretion of riboflavin by the same 18 patients was within the normal limits, the range being from 175 to 1300 gamma per day, with an average of 622 gamma per day.

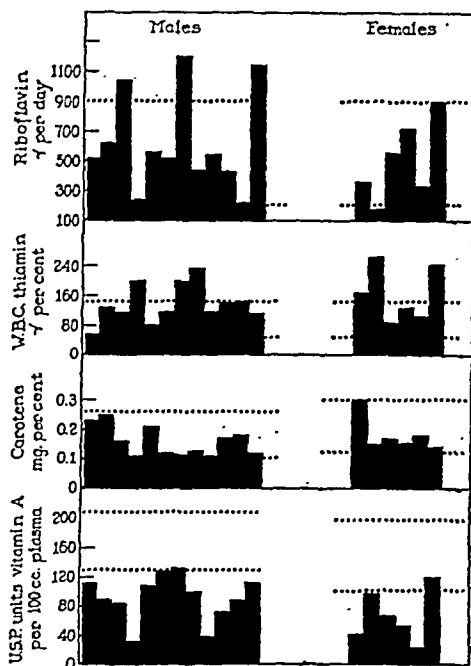


FIG. 7. PLASMA VITAMIN A AND CAROTENE LEVELS, WHITE CELL THIAMIN AND URINARY RIBOFLAVIN LEVELS OF 19 PATIENTS WITH CANCER OF THE GASTRO-INTESTINAL TRACT

Only 1 patient had an abnormally low daily output of riboflavin in the urine (175 gamma per day), but she had a normal plasma carotene of 0.15 mgm. per cent and also an exceptionally high white cell concentration of thiamin (224 gamma per 100 cc.).

From these findings, there was no evidence that the diet had been generally inadequate in the 18 patients investigated whose low plasma levels of vitamin A were associated with normal levels of carotene. One could not be entirely satisfied, however, that the normal values used for comparison were valid as controls for this group of patients. Since the normal individuals examined differed widely in age, race, and economic background from the patients, it seemed possible that

these factors, rather than the disease from which they suffered, might be responsible for the low levels of vitamin A in the plasma of the patients. It was important to establish, therefore, whether individuals of the same age, race, and economic status as the patients also would have low levels of plasma vitamin A.

As a preliminary step, it seemed feasible to test groups of patients similar in social and economic background to those with gastro-intestinal cancer. If levels of vitamin A as low as those in the patients with gastro-intestinal cancer were found in these individuals, a further check would be provided.

Of 20 patients with leukoplakia of the oral mucosa, however, only 3 (15 per cent) showed such levels, and 2 of these 3 had been on grossly deficient diets. None of the 20 was known to have syphilis or associated cancer. Similarly, of the 13 patients with atrophic gastritis, none showed abnormally low plasma levels of vitamin A. All of the 13 had symptoms referable to their disease, and 4 had been on grossly inadequate diets.

No further proof seemed necessary, therefore, to establish the fact that race, age, and economic background were not responsible for the low levels in the patients with gastro-intestinal cancer. These findings, furthermore, indicate that patients bearing supposedly precancerous lesions, namely oral leukoplakia and atrophy of the gastric mucosa, do not have low plasma levels of vitamin A.

From the evidence at hand, it was only possible to conclude that the patients with gastro-intestinal neoplasm were in some way unable to distribute normally the vitamin A taken in with the diet. Such an abnormality might be due simply to the presence of a lesion which interfered with the absorptive power of the gastro-intestinal tract, quite irrespective of its malignant nature. Were this the case, patients with benign disorders of the intestinal tract marked by persistent vomiting or diarrhea should show similarly low levels of plasma vitamin A.

Fourteen patients with benign lesions were examined. Eight had peptic ulcers, 2 severe ulcerative colitis, 2 had long-standing active sprue and 2 mucous colitis. The 8 patients with peptic ulcer had had nausea, vomiting and weight loss for

several months at the time of examination. The remaining 6 had had diarrhea and weight loss for from 1 to 10 years. There were 6 males and 8 females in the group. The 6 males had plasma levels of vitamin A which ranged from 102 to 212 U.S.P. units per 100 cc., and averaged 180 U.S.P. units per 100 cc., or slightly above the

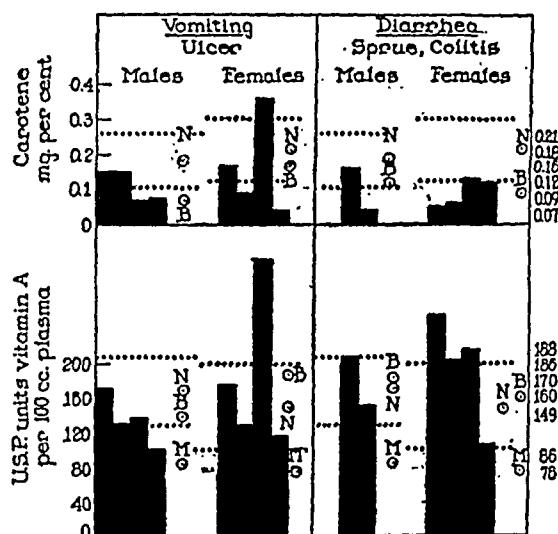


FIG. 8. PLASMA VITAMIN A AND CAROTENE LEVELS OF 14 PATIENTS WITH BENIGN LESIONS OF THE GASTRO-INTESTINAL TRACT

normal average for males. The 8 females had plasma vitamin A levels which ranged from 104 to 320 U.S.P. units per 100 cc. and averaged 190 U.S.P. units, a figure somewhat higher than the normal average for females. Of the 14 patients, only 2 males had plasma vitamin A concentrations below the normal range (Figure 8).

#### 1. Effect of administration of vitamin A on plasma levels of that substance in patients with gastro-intestinal cancer

From the results of the study described above, it appeared probable that the low levels of vitamin A in the plasma of the patients with gastro-intestinal cancer were due neither to poor dietary intake nor to functional disturbance of the gastro-intestinal tract, but rather to lack of absorption or to some metabolic disturbance which prevented the vitamin A ingested from appearing in the plasma.

In order to investigate this possibility further, the effect of administration of vitamin A on the plasma levels of these patients was compared with

the effect of the administration of the same amounts of vitamin A to patients with similarly low plasma levels associated with other disorders. Eight patients with low plasma vitamin A levels associated with lesions other than gastro-intestinal tract cancer were given from 50,000 to 150,000 units daily of vitamin A for from 6 to 20 days. The vitamin was administered parenterally to 5 and orally to 3. Three of the patients had well-advanced cancer of the pancreas, 2 had hepatic cirrhosis, 1 pyloric obstruction secondary to a duodenal ulcer, 1 chronic myeloid leukemia, and 1 an osteogenic sarcoma. In every one the ad-

to the patients with low plasma levels associated with lesions other than gastro-intestinal tract cancer. The vitamin was administered orally to 1 patient, parenterally to 6, and both orally and parenterally to 1. In 5 of the 8 patients the low vitamin A levels were associated with normal plasma concentrations of carotene. Only 1 patient previously had been on a deficient diet. In only 2 of the 8 patients did the plasma level of vitamin A rise following the administration of that substance. Neither of these had been on grossly deficient diets, and both had plasma carotene levels within the normal range. In the other

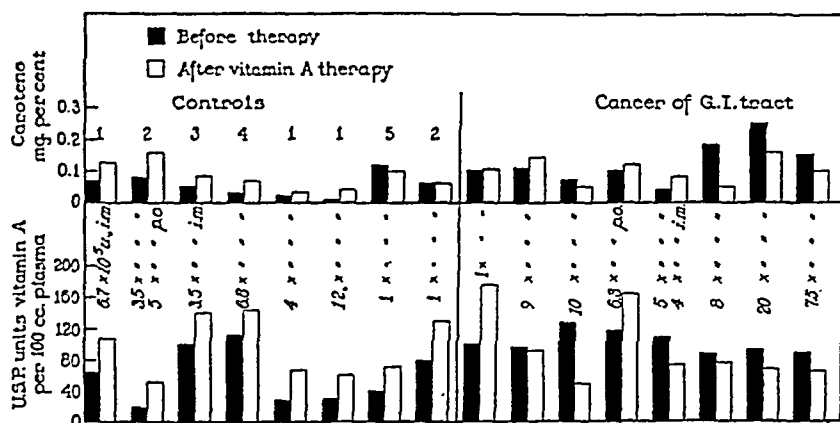


FIG. 9. EFFECT OF VITAMIN A SUPPLEMENT ON THE LEVELS OF VITAMIN A AND CAROTENE IN THE PLASMA OF A CONTROL GROUP AND OF THOSE WITH CANCER OF THE GASTRO-INTESTINAL TRACT

1. Cancer of pancreas. 2. Cirrhosis. 3. Peptic ulcer. 4. Leukemia. 5. Osteogenic carcinoma.

ministration of vitamin A was followed by a rise in the plasma levels of from 40 to 180 per cent above the original levels (Figure 9). The average rise was 90 per cent. Further evidence that the amount of vitamin administered was sufficient to raise a level, low because of simple deficiency, is provided by the studies of Steininger (12) and of Murrill (13) who demonstrated that the low plasma levels of vitamin A of normal individuals on vitamin A-deficient diets could be restored to normal by the administration of from 20,000 units (12) to 400,000 units (13) of vitamin A.

In contrast to the findings just described were the results of a similar study in patients bearing cancer of the gastro-intestinal tract. Eight male patients with low plasma concentrations of vitamin A were given the same amounts of the vitamin, over periods of from 7 to 20 days, as were given

6 patients the plasma vitamin A levels after administration were from 5 to 33 per cent less than they had been before, and the average fall was 28 per cent (Figure 9).

In neither the control group nor the group with gastro-intestinal cancer did the administration of vitamin A effect any consistent change in the concentration of carotene in the plasma.

It appeared, then, that in the patients with gastro-intestinal tract cancer some specific disorder was present which prevented the normal distribution of vitamin A by the patients, even though amounts were made available which could raise the low levels in patients with other disorders. However, it was also possible that the rate of destruction or excretion of the vitamin was abnormally high. No methods for the investigation of these possibilities were at hand.



## 2. Plasma levels of vitamin A in patients who had had cancer of the gastro-intestinal tract removed

In order to determine whether or not the low plasma levels of vitamin A were contingent upon the actual presence of cancer in the organism, the concentration of the vitamin was determined in the plasma of 19 patients who had had gastro-intestinal cancer surgically removed. Eleven of the patients were males and 8 females. Twelve originally had had cancer of the stomach, and 7 had cancer of the large bowel. The patients were examined from 3 months to 10 years after operation, and at the time of examination were believed to be free of neoplastic disease. Of the 19 patients, 3 had been on grossly deficient diets for at least 4 months before the vitamin A levels were determined.

Of the 11 male patients, 4 had plasma vitamin A levels below the normal range, but the average was 176 U.S.P. units per 100 cc., only slightly more than the average for normal males (170.3 U.S.P. units). Of the 8 females, only 2 had a plasma vitamin A level below the normal range, and the average was 145 U.S.P. units per 100 cc., a figure slightly less than the average found in normal females (149.1 U.S.P. units) (Figure 10).

It would appear, then, that the incidence of low plasma levels of vitamin A in patients who have had gastro-intestinal cancer surgically removed is considerably less than in patients with the cancer still present.

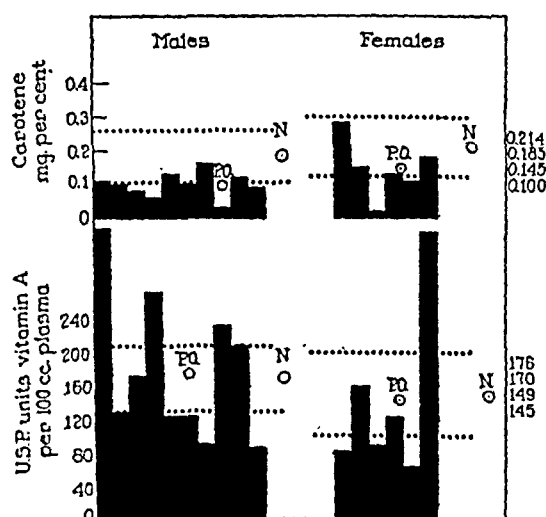


FIG. 10. PLASMA VITAMIN A AND CAROTENE LEVELS IN PATIENTS WHO HAD CANCER OF THE GASTRO-INTESTINAL TRACT REMOVED

## 3. Plasma levels of vitamin A in patients with malignant diseases other than gastro-intestinal cancer

It was next important to discover whether low plasma vitamin A levels were confined to individuals with cancer of the gastro-intestinal tract and, accordingly, a group of patients with malignant neoplasms of other types was examined.

Of 48 patients with leukemia, the average plasma vitamin A level of 34 males was 119 U.S.P. units, and the average vitamin A level of 14 females was 113 U.S.P. units. Of the 48 patients, 24, or 50 per cent, had plasma levels below the normal range. Of 21 patients with Hodgkin's disease, the average plasma vitamin A level of 11 males was 63.3 U.S.P. units, and the average level of the 10 females was 75.9 U.S.P. units. Of the whole group, 18, or 84 per cent, had levels below the normal range. Of 9 patients with cancer of the head of the pancreas, the average for the 6 males was 115 U.S.P. units, and for the 3 females 101 U.S.P. units. Five of the 9 patients had levels below the normal range. Of 6 patients with bone sarcoma, 3, all males, had plasma levels of vitamin A below the normal limits.

In sharp contrast were the findings in patients with mammary and oral cancer. Only 1 of the 20 women with cancer of the breast, and none of the 8 patients with intra-oral neoplastic disease, had plasma levels below the normal range.

These findings are of interest, since they indicate that low levels of vitamin A in the plasma are not specific for gastro-intestinal cancer but also may be found associated with other forms of malignant disease.

## C. The effect of the administration of substances containing other vitamins on the plasma levels of vitamin A in patients with gastro-intestinal cancer and with other diseases

Since the administration of vitamin A in amounts adequate to raise the low levels of the vitamin in the plasma of patients not bearing gastro-intestinal cancer had only an irregular effect on the plasma levels in patients with this disease, the effect of the administration of other vitamins was studied.

### 1. Yeast

As a part of a general therapeutic regime to prepare the patients for operation, 90 grams of Fleischmann's 20-40 granular Brewer's yeast were fed daily, over periods of from 4 to 32 days, to 17 patients with gastro-intestinal cancer. This yeast was shown to be free of both vitamin A and carotenoids both by the Carr-Price reaction and by biological assay. One of the 17 patients previously had been given 750,000 U.S.P. units of vitamin A parenterally, and that amount of the vitamin had not elevated the originally reduced plasma level.

All of the 17 patients, after yeast therapy without vitamin A, showed a substantial elevation of their plasma levels of vitamin A. Fourteen of the 17 patients at the outset had plasma levels below the normal range. The average rise of vitamin A in the plasma of the 17 patients was 215 per cent, and the range was from 12 to 835 per cent (Figures 11 and 12).

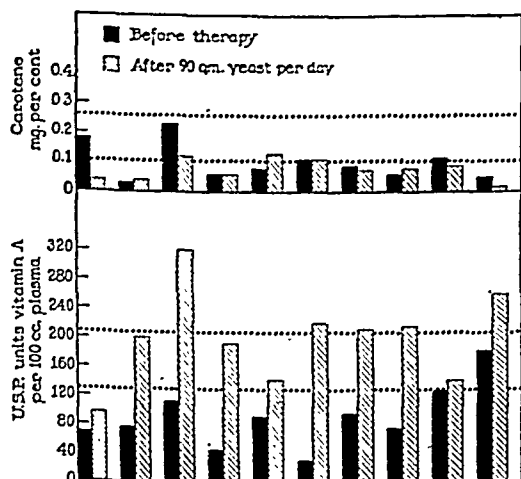


FIG. 11. PLASMA VITAMIN A AND CAROTENE LEVELS OF MALES WITH CANCER OF THE GASTRO-INTESTINAL TRACT BEFORE AND AFTER YEAST THERAPY

In contrast to the effect on the plasma concentration of vitamin A was the lack of any consistent change in the plasma levels of carotene after the administration of yeast. In 6 of the 17 treated patients the carotene rose, in 7 it fell, and in 4 there was no change whatsoever.

In 4 instances, after the yeast had effected a rise in the levels of vitamin A in the plasma, the therapy was discontinued. The withdrawal was

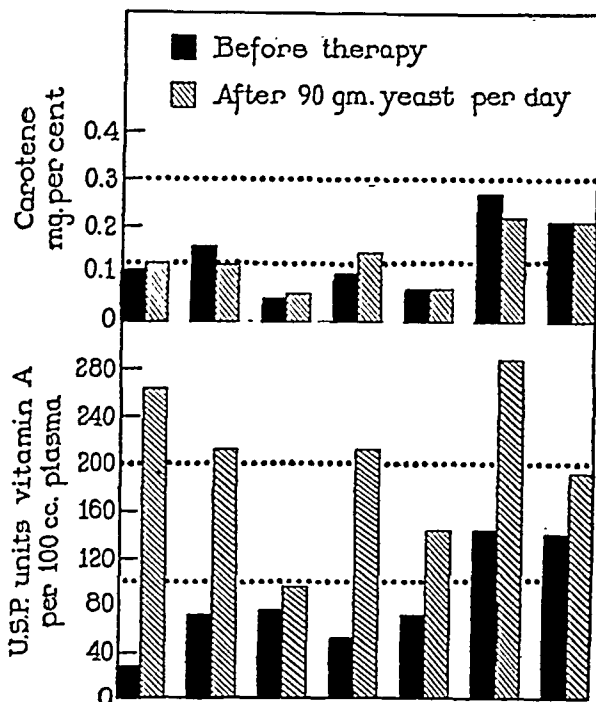


FIG. 12. PLASMA VITAMIN A AND CAROTENE LEVELS OF FEMALES WITH CANCER OF THE GASTRO-INTESTINAL TRACT BEFORE AND AFTER YEAST THERAPY

followed in each instance by a drop in the vitamin A levels. The course of 1 of these patients, G. B., is graphically depicted in Figure 13. In this patient a second rise was obtained when the treatment was reinstituted.

Similar elevation of low vitamin A levels to the normal range followed the administration of

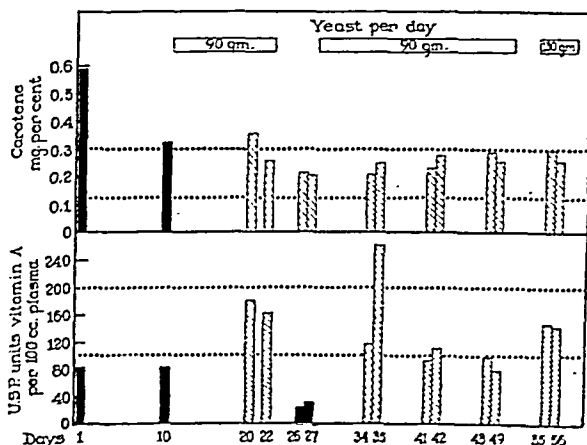


FIG. 13. PLASMA VITAMIN A AND CAROTENE LEVELS OF PATIENT G. B., 9, WITH CANCER OF THE COLON BEFORE AND AFTER YEAST THERAPY

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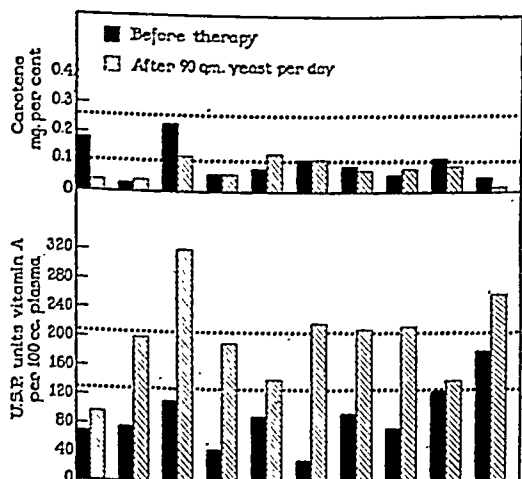


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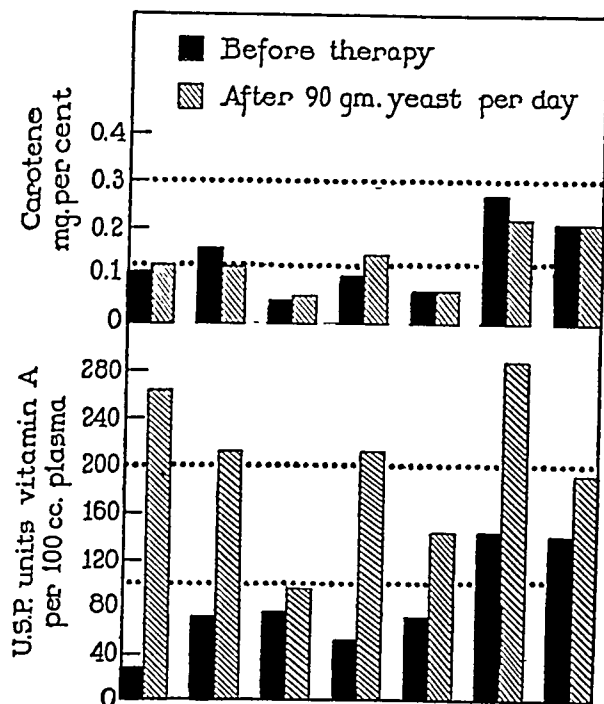


FIG. 12. PLASMA VITAMIN A AND CAROTENE LEVELS OF FEMALES WITH CANCER OF THE GASTRO-INTESTINAL TRACT BEFORE AND AFTER YEAST THERAPY

followed in each instance by a drop in the vitamin A levels. The course of 1 of these patients, G. B., is graphically depicted in Figure 13. In this patient a second rise was obtained when the treatment was reinstituted.

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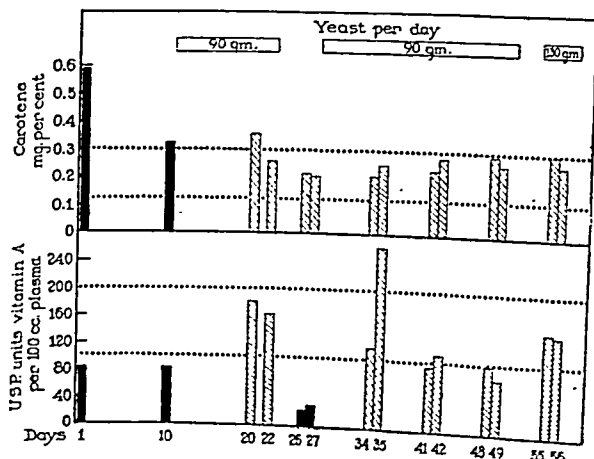


FIG. 13. PLASMA VITAMIN A AND CAROTENE LEVELS OF PATIENT G. B., ♀, WITH CANCER OF THE COLON BEFORE AND AFTER YEAST THERAPY

yeast to 13 patients who had such diseases as hepatic cirrhosis, retinitis pigmentosa, cholecystitis, polyposis coli, and cancer of the head of the pancreas. The moderately reduced level of vitamin A in the plasma of a patient with a peptic ulcer was also restored to the normal range after the patient was fed yeast. There was no consistent effect on the plasma carotene of this group (Figure 14).

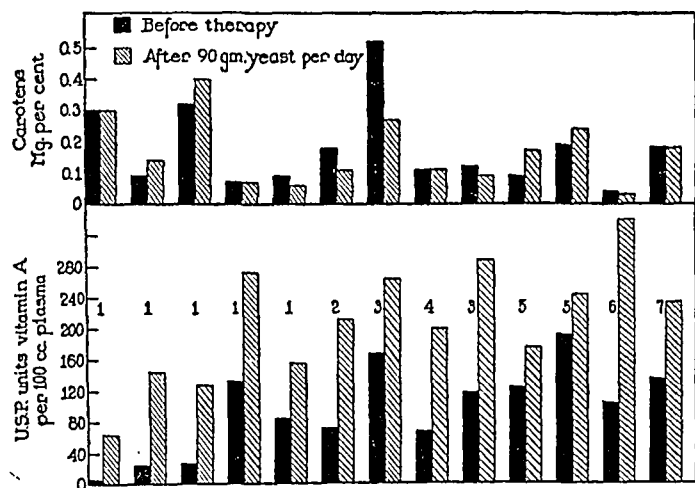


FIG. 14. EFFECT OF YEAST ON PLASMA VITAMIN A AND CAROTENE LEVELS IN PATIENTS WITH DISEASES OTHER THAN CANCER OF THE GASTRO-INTESTINAL TRACT

1. Cirrhosis. 2. Nyctalopia. 3. Duodenal ulcer. 4. Cholecystitis. 5. Gastric ulcer. 6. Cancer of pancreas. 7. Polyposis.

On the other hand, no elevation of reduced plasma levels of vitamin A followed the administration of yeast to 2 patients with Hodgkin's disease and to 3 patients with chronic leukemia. The normal plasma vitamin A and carotene levels of 2 normal individuals also were unaffected after the administration of 90 grams of yeast each day for 1 week.<sup>4</sup>

## 2. Lipocaic and choline

The ability of yeast to raise the plasma levels of vitamin A in patients with gastro-intestinal tract cancer was considered to be due to one or some combination of the following causes: (1) The yeast aided the absorption of ingested carotenoids through the intestinal wall. (2) It contained a substance with lipotropic activity which caused the release into the blood stream of fat-

soluble vitamin A and other fat-soluble compounds from the liver or from other fat depots of the body. (3) The yeast corrected some abnormality which prevented the normal deposition of the vitamin.

The first possibility has been covered by the observation that even the parenteral administration of vitamin A usually did not elevate the low levels of the vitamin in the plasma of patients with gastro-intestinal cancer.

To examine the second possibility, two materials with known lipotropic activity were administered to patients. The first material was lipocaic,<sup>5</sup> and its use was suggested by Dr. Charles Best of Toronto. It is a pancreatic extract prepared after the method of Dragstedt (14). The second lipotropic substance tested was choline chloride.<sup>6</sup>

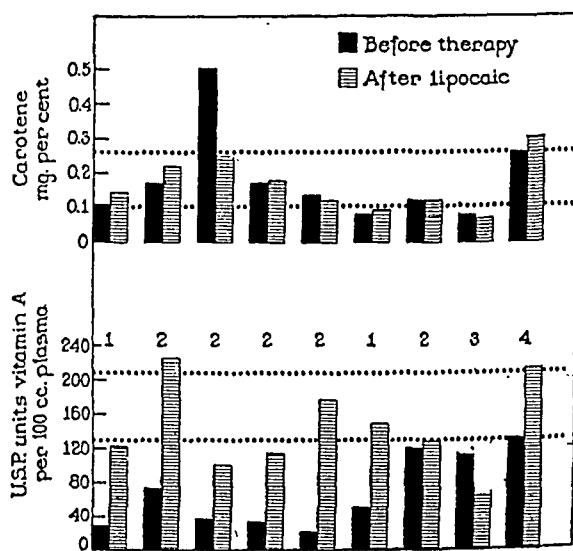


FIG. 15. PLASMA VITAMIN A AND CAROTENE LEVELS OF MALES BEFORE AND AFTER LIPOCAIC

1. Cancer of colon. 2. Cancer of stomach. 3. Cancer of pancreas. 4. Normal.

(a) *Lipocaic*. Two patients with rectosigmoid cancer and 5 with gastric cancer were fed 5 grams daily of lipocaic for from 4 to 18 days. All had low plasma levels of vitamin A before therapy was instituted, and in 6 of the 7 cases, therapy effected substantial, sustained increase of the concentration of the vitamin in the plasma. The seventh case showed only a slight increase after

<sup>5</sup> Supplied through the courtesy of the Eli Lilly Company.

<sup>6</sup> Supplied through the courtesy of the S. M. A. Corporation.

<sup>4</sup> The authors are indebted to the Standard Brands, Inc., for supplying the yeast used in this investigation.

therapy. The average rise in the plasma of the 7 patients treated with lipocaic was 270 per cent, and the range was from 6 to 710 per cent (Figure 15). The results in 2 of these patients are shown in Figures 16 and 17.

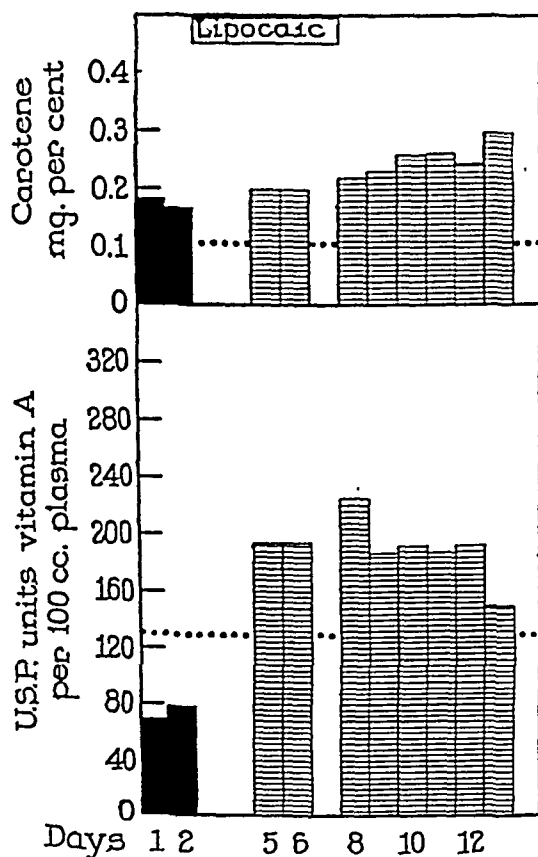


FIG. 16. PLASMA VITAMIN A AND CAROTENE LEVELS OF PATIENT S. T., ♂, WITH CANCER OF THE COLON BEFORE AND AFTER LIPOCAIC THERAPY

Five grams of lipocaic were fed each day for 6 days to 2 normal individuals. After the administration, the plasma levels of vitamin A rose in one individual from 126 to 212 U.S.P. units, and in the other from 140 to 215 U.S.P. units.

Five grams of lipocaic were administered each day for 4 days to a patient with cancer of the pancreas. After the administration of this substance, his plasma level of vitamin A fell from 112 to 64 U.S.P. units.

(b) *Choline chloride*. The lipotropic effect of this substance now has been demonstrated by several workers (15, 16).

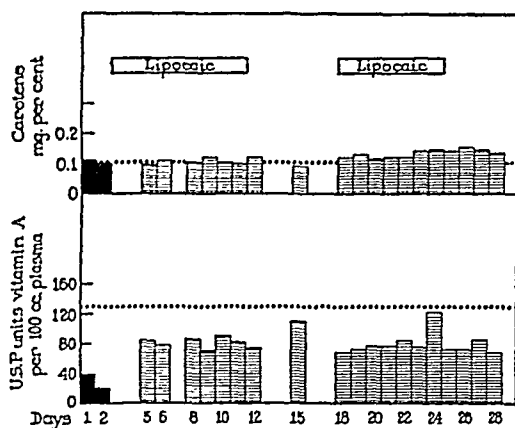


FIG. 17. PLASMA VITAMIN A AND CAROTENE LEVELS OF F. C., ♂, BEFORE AND AFTER LIPOCAIC THERAPY

Six patients with gastric cancer and 1 with rectal cancer were fed 1.5 grams of choline chloride each day for 3 days. Five of the 6 patients had abnormally low plasma vitamin A levels when the administration of choline was begun. In all 6 patients the therapy effected substantial increases in the plasma levels of the vitamin. The average rise after choline chloride was 73 per cent, and the range was from 30 per cent to 140 per cent (Figure 18).

One and a half grams of choline chloride were fed each day for from 3 to 5 days to 5 patients with such diseases as retinitis pigmentosa, peptic ulcer, cancer of the bronchus, sarcoma of the

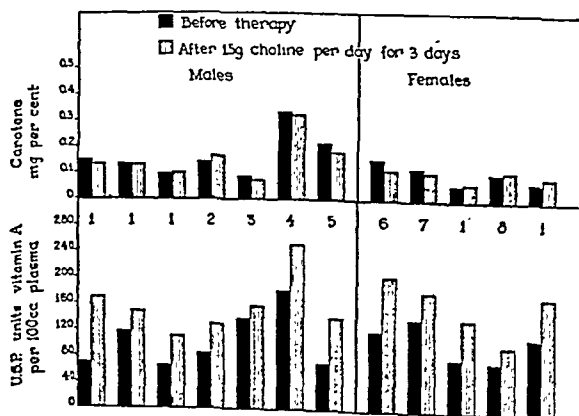


FIG. 18. PLASMA VITAMIN A AND CAROTENE LEVELS IN PATIENTS BEFORE AND AFTER CHOLINE THERAPY

1. Carcinoma of stomach. 2. Carcinoma of bronchus. 3. Hemolytic anemia. 4. Retinitis pigmentosa. 5. Carcinoma of rectum. 6. Sarcoma of stomach. 7. Peptic ulcer. 8. Gastritis.

stomach, and hemolytic anemia. In every instance the administration of choline chloride effected a rise in the plasma levels of vitamin A. The average rise was 98 per cent (Figure 18), and the range was from 15 per cent to 300 per cent.

Nine normal individuals also were given 1.5 grams of choline chloride each day for 3 days. After the administration of this substance, the vitamin A levels in the plasma of all 9 individuals increased from 6 per cent to 90 per cent. The average rise was 46 per cent (Figure 19).

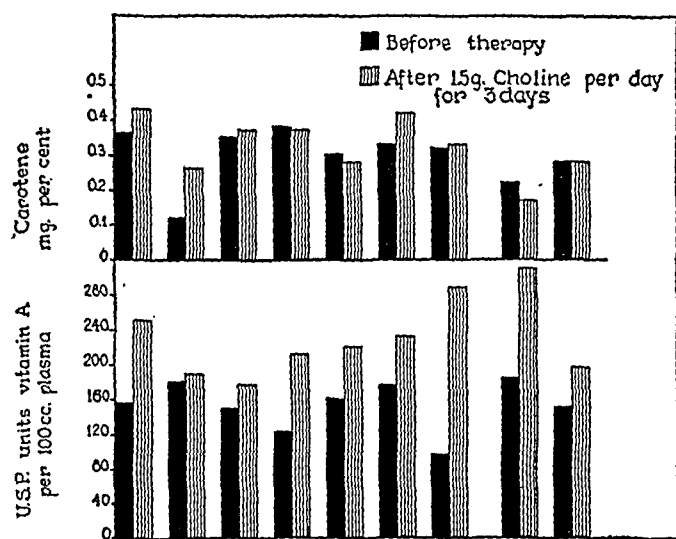


FIG. 19. PLASMA VITAMIN A AND CAROTENE LEVELS IN NORMAL ADULTS BEFORE AND AFTER CHOLINE

It appears from the evidence that the effect of yeast, and that of lipocaic and choline, is somewhat similar as far as the plasma levels of vitamin A in the patients studied are concerned. Further investigation will be required to establish the identity, or lack of identity, of the effective constituents of the three materials tested.

Experiments are now in progress to test the third possible action of the yeast, namely that the administration of the yeast corrected an abnormality which prevented normal storage of the vitamin A. The vitamin A and carotene contents of liver biopsies of patients with and without gastro-intestinal cancer are now being determined. Half of these patients are fed yeast or vitamin A previous to laparotomy.

#### DISCUSSION

All of the vitamin A determinations in this communication were based on the Carr-Price reaction. This reaction is not specific for vitamin

A alone, since certain plant pigments and carotene are known to give a blue color reaction with antimony trichloride (17). However, the Carr-Price reaction is the most satisfactory chemical method for the quantitative estimation of vitamin A and carotene (18), and it is generally accepted that the amount of blue color produced by the reaction of the vitamin with antimony trichloride is proportional to the amount of vitamin A present (7). Koehn and Sherman (7) found that values arrived at by biochemical assays were in excellent agreement with the photoelectric measurements of the Carr-Price reaction products of vitamin A in cod liver oil. From their studies they obtained a factor of 2120 with which to convert E (1 per cent, 1 cm.) into U.S.P. units of vitamin A per gram of oil.

Barthen and Leonard (19), furthermore, found that the biological assay of vitamin A in fish oils gave values which checked closely with those obtained by direct spectroscopic measurements of the same samples. These workers arrived at a conversion factor of 2222.

Baxter and Robeson (20) have prepared crystalline vitamin A. Spectrophotometric measurements of the crystalline vitamin itself and of the blue Carr-Price reaction product of it gave values within 3 per cent of each other. These authors compared the vitamin A potency of fish oil preparations both by bio-assay and by spectrophotometry, and calculated a factor of 2100 by which one could convert the spectrographic extinction coefficient into U.S.P. units. This last conversion factor has been confirmed (21).

There have been no reports in the literature of direct spectrophotometric measurements of those substances in blood plasma which give the blue color with the Carr-Price reagent. At our suggestion, Dr. Embree and Dr. Shantz of the Distillation Products, Inc., analyzed a sample of plasma for its vitamin content.<sup>7</sup> This sample was first assayed in this laboratory by the Carr-Price reaction, and was found to contain 75 U.S.P. units of the vitamin. Spectrophotometric measurements of the antimony trichloride reaction product then indicated that the sample contained 72 U.S.P. units.

<sup>7</sup> This work was done by the Vitamin Distillation Corporation through the courtesy of Dr. N. D. Embree.

The same sample of plasma was treated by the technique of Embree (22) to cyclize any vitamin A which may have been present. The plasma treated by their technique showed an absorption spectrum after treatment which was typical of cyclic vitamin A, and the amount of cyclic vitamin A formed (72 U.S.P. units) corresponded very closely with the amount of vitamin A assumed to be present on the basis of the Carr-Price reaction previously performed on the same sample of plasma; namely, 75 U.S.P. units.

Since the various conversion factors obtained by different groups of investigators have checked so closely, and since the cyclic vitamin A measured in the plasma could have been formed only from preexisting vitamin A, it is evident that the interpretation of the results of the Carr-Price technique used in the studies here reported rests on a sound basis.

It was important to determine whether or not the apparent increase of vitamin A in the plasma, effected by the administration of yeast and lipocaic, was due to vitamin A or other carotenoids. When the Carr-Price technique was applied to yeast and lipocaic, the final extracts gave no blue color. It therefore seemed unlikely that the increase of the Carr-Price reactants of the plasma of patients treated with these materials was due to the administration of any carotenoids. Choline chloride does not give the Carr-Price reaction.

Both yeast and lipocaic contain fractions which can reduce the fat content of the fatty liver of animals treated with biotin (23). The fatty livers of pancreatectomized animals, controlled with insulin and glucose, and of animals on high fat diets, can be prevented, and the fat content of the already pathological liver reduced, when the animals are fed lipocaic (24, 25), or yeast (26). Choline chloride also can prevent the deposition of fat into the livers of animals on high fat-low protein diets (27) and reduce the lipid content of the fatty liver (28). Choline exists in both yeast (29) and lipocaic (30) and therefore the lipotropic effects of those materials could have been due to their content of choline.

It is possible, therefore, that the lipotropic properties of yeast, lipocaic and choline, which demobilize lipoids from the liver, might be responsible for the increase in the content of fat-soluble vitamin A in the plasma after the administration

of these lipotropic substances. This possibility is based on the assumption that the lipoids in the fatty liver may withhold from the blood the fat-soluble vitamins—an assumption as yet unproved by experiment. Should this be the explanation of the increased levels of vitamin A in the plasma after the yeast and lipocaic therapy, then there must come a time when continued treatment will deplete all the hepatic store of vitamin A. Continued therapy, therefore, should be unable to maintain the increased plasma concentration of the vitamin. This stage may have been reached in the case of G. B. (Figure 13) cited in the previous section. The concentration of vitamin A had been increased in the plasma of that patient by feeding 90 grams of yeast per day, but after 20 days of yeast therapy the plasma level of the vitamin once again fell to near its original value.

Although the Carr-Price reactants of the plasma appear to be entirely vitamin A, it would be important, nevertheless, to determine whether or not the increase of plasma vitamin A after yeast therapy represents an apparent or true increase. Plans for such an experiment are in progress in collaboration with Dr. Embree of the Vitamin Distillation Corporation.

It also would be interesting to correlate the plasma levels of the Carr-Price reactants with an improvement of a physiologic function dependent upon vitamin A. Both good and bad correlations of the plasma levels of vitamin A with visual dark adaptation have been reported (31, 32). A recent study which employed the Hecht adaptometer (33) concluded that the plasma vitamin A level of an individual was not a good index of his ability to effect dark adaptation.

The explanation for the observation that patients with gastro-intestinal cancer have low plasma levels of vitamin A was considered at first to be an insufficient intake of carotenoids. This was thought to be due possibly to a deficiency of carotenoids in the diet or to malabsorption of carotenoids from the gastro-intestinal tract. However, as previously indicated, deficient diets or a deficient intake of carotenoids could not explain the low plasma vitamin A levels in the 51 patients with gastro-intestinal cancer. This was demonstrated by the following observations: (1) Seventy-five per cent of the patients had satisfactory dietary histories. (2) Only 15 per



cent of a control group of patients with oral leukoplakia, and none of a control group with atrophic gastritis who had been on a similar diet, had reduced levels of plasma vitamin A. (3) Fifty-five per cent of the 51 patients had normal plasma levels of carotene. (4) Of the 28 patients who had low plasma levels of vitamin A and normal plasma carotene levels, 18 were examined further for thiamin or riboflavin deficiency. None of the 18 were deficient in thiamin and only 1 was deficient in riboflavin. (5) Of great importance was the observation that the parenteral administration of large amounts of vitamin A was unable to raise the plasma level of the vitamin in 6 of 8 patients with gastro-intestinal cancer, but was able to do so in all of 8 patients with vitamin A deficiency who were used as controls. This latter group included 6 patients with malignant neoplastic disease other than gastro-intestinal cancer and 2 with non-neoplastic disorders. It has been demonstrated by Steininger (12) and by Murrill (13) that normal individuals on low vitamin A diets develop low levels of the vitamin in their plasma. The administration of from 20,000 to 400,000 units of vitamin A promptly restored to normal the plasma levels of the vitamin in the normal individuals.

It is unlikely that malabsorption of fat-soluble vitamins could have been responsible for the low levels of vitamin A in the plasma of patients with gastro-intestinal cancer. Only 6 of the 51 patients presented either had diarrhea or suffered from persistent vomiting. It is true that in those 6 patients the diarrhea or vomiting might have prevented adequate absorption of vitamin A from their gastro-intestinal tracts. If this were the reason for the low vitamin A concentration in the plasma of those 6 patients, then low concentrations of the vitamin should have been expected to exist in the plasma of other patients bearing benign gastro-intestinal lesions and who had a comparable degree of diarrhea or vomiting. However, of 14 patients with benign gastro-intestinal lesions, 6 had severe diarrhea and 8 had suffered from persistent vomiting, but only 2 of these 14 had a plasma vitamin A level below the normal range.

It was evident, then, that patients with gastro-intestinal cancer could have low levels of vitamin A in their plasma despite their adequate ingestion

and absorption of the vitamin. Then, could the low plasma levels of vitamin A in these patients be due to an inability to store the vitamin or to convert it from its precursor carotenoids?

Under normal conditions about 90 per cent of the vitamin A in the human body is stored in the liver (34, 35). It has been demonstrated that damaged livers have difficulty in storing other substances; namely, glycogen (36), labile protein (37), and the anti-pernicious anemia principle (38). It is thus plausible that the damaged liver might no longer be able to store vitamin A. It previously has been pointed out that the hepatic store of vitamin A in patients with portal cirrhosis and with acute or chronic liver atrophy is considerably reduced (39 to 42). Undoubtedly, the liver is intimately concerned with the metabolism of vitamin A since, not only does it control the distribution of the vitamin in various parts of the organism (34), but it also converts carotene through an enzymatic reaction into the vitamin (43, 44). It appears that, as other hepatic functions are lost in the damaged liver, this conversion of carotene into vitamin A also is impaired (45, 46).

It was important, therefore, to ascertain whether or not patients bearing cancer of the gastro-intestinal tract have any anatomical or physiological derangement of their livers. The most likely explanation for hepatic derangement in these patients would be metastatic replacement of liver tissue by the cancer. However, of the 51 patients, the livers of 37 were examined at laparotomy and of these, 26 had no gross evidence of metastatic involvement. The cancerous infiltration into the livers of the other 11 patients was minimal. There was no higher incidence of low plasma vitamin A levels among the patients with metastases to the liver than among those whose livers were free of cancer.

Nevertheless, the patients with gastro-intestinal cancer presented in this communication did have evidence of hepatic insufficiency. This insufficiency was indicated by the fact that 2 or more of 8 liver functions which were tested in each patient of this group were discovered to be abnormal. The details of this study will form the subject of a subsequent report.

To date, 19 patients have been studied who have had successful resection of gastro-intestinal can-

cer from 3 months to 10 years ago. Fourteen of these patients had plasma levels of vitamin A within normal limits and considerably less evidence of hepatic dysfunction than did the patients bearing gastro-intestinal cancer.

The hepatic dysfunction found to exist in patients with cancer of the gastro-intestinal tract might account for a derangement in the metabolic processes which involve vitamin A formation and storage. It is impossible to state definitely, as yet, whether or not the hepatic dysfunction and the low plasma level of vitamin A found in the patients with gastro-intestinal cancer are dependent on the presence of cancer in the organism, but this appears probable from the evidence.

#### SUMMARY AND CONCLUSIONS

1. The levels of vitamin A in the plasma of patients with gastro-intestinal cancer were found to be below the normal range in 86 per cent of the patients examined.

2. The probable explanation for the low plasma levels of vitamin A is thought to be (a) an inadequate ingestion and absorption of the vitamin, (b) an hepatic dysfunction as concerns the storage of vitamin A or its formation from carotene.

3. It has been shown that in a proportion of the patients studied, a dietary deficiency or malabsorption of the vitamin could not explain the low plasma vitamin A values.

4. Patients who have had successful resection of gastro-intestinal cancer have a much lower incidence of reduced plasma levels of vitamin A than do the patients in whom gastro-intestinal cancer is still present.

5. The administration of yeast and lipocaic, themselves free of carotenoids, raised the reduced plasma levels of vitamin A in patients with gastro-intestinal cancer. This property of the yeast and lipocaic could have been due to choline chloride and perhaps to other lipotropic substances contained in these materials.

6. A high incidence of reduced vitamin A levels also was found in the plasma of patients with other malignant diseases; namely, with lymphomas, cancer of the head of the pancreas, and bone sarcoma.

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# FRACTIONATION OF SERUM PROTEINS IN HYPERPROTEINEMIA, WITH SPECIAL REFERENCE TO MULTIPLE MYELOMA

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Investigation of the serum proteins in disease is handicapped by lack of clinically applicable methods for resolving the complex system of proteins present in blood serum into homogeneous components. The only fractionation now possible is into *groups* of proteins having like solubility characteristics or electrophoretic mobilities or sedimentation constants (depending upon the method employed) but varying in other properties and in composition (1). However, these methods, if rigidly standardized, give reproducible results and, in certain diseases, characteristic divergencies from normal patterns. The separations therefore, though arbitrary, have at least empirical value.

The present study deals with the empirical application of Howe's method (2) and the electrophoretic technique (3, 4) to fractionation of the serum proteins in various diseases presenting hyperproteinemia. Multiple myeloma is of special interest in this connection. Not only is the incidence and degree of hyperproteinemia unusually high (5, 6, 7, 8), but there is extraordinary variability in the composition of the protein increment (7, 9), as indicated by fractional precipitation with neutral salts (5, 10, 11, 12), by electrophoresis (4, 13), and by ultracentrifugation (13). The significance of these peculiarities has not been made clear.

To provide a common basis for consideration of our findings in hyperproteinemia, we have first cited results obtained in normal adult subjects. Serum protein partitions in a variety of chronic infections and in cirrhosis are then recorded to illustrate the uniform plan in distribution of globulin subfractions which characterizes hyperproteinemia of all such etiology. Some of our cases with elevated serum protein levels due to multiple myeloma likewise showed this conventional fractional distribution. Others, however,

presented certain distinctive anomalies in both the Howe and electrophoretic patterns, which were found to be due in large part to Bence-Jones proteinemia and to be of value in diagnosis.

## METHODS

Blood samples were withdrawn without prolonged tourniquet stasis.

Total nitrogen was determined in 0.5 ml. samples of serum, made up to 25 ml. with 0.9 per cent NaCl solution in a volumetric flask. Triplicate 4 ml. aliquots were digested in 100 ml. Pyrex Kjeldahl flasks, then steam-distilled in a modified Pregl micro-Kjeldahl apparatus into 5 ml. saturated boric acid (14) containing methyl red (recrystallized) indicator. Titration was carried out with N/70 HCl, matching with a control containing 5 ml. boric acid-indicator mixture plus sufficient blank distillate to approximate the volume of the unknown. Non-protein nitrogen was determined by the Folin-Wu method.

The Howe technique has been carried out with precautions since November, 1937, to minimize the filtration error (15). The albumin fraction was determined in 1.0 ml. samples of serum. Thirty ml. of a 22.2 per cent solution of sodium sulfate (Merck anhydrous nitrogen-free) were added in an incubator room maintained at 37°, where the mixture remained (with the usual precautions) for 3 hours to overnight. Nine cm. Number 50 Whatman paper was used and the first filtrate, approximately 2/5 of the total volume, was discarded. Nitrogen was determined in triplicate 3 ml. aliquots of the last filtrate by the procedure indicated. The total serum globulin fraction was estimated by difference.

The "euglobulin" fraction was determined in 0.5 ml. samples of serum to which were added 15 ml. of 14 per cent sodium sulfate at 37°. Filtration was carried out with 7.0 cm. Number 50 Whatman filter paper. The first portion of the filtrate, about 1/3 of the total volume, was discarded. Nitrogen was determined in triplicate 3 ml. aliquots of the last portion of filtrate by the procedure indicated. The "pseudoglobulin I" fraction was estimated in 0.5 (or, if available, 1.0) ml. samples of serum in the same way, using 18 per cent sodium sulfate. The "pseudoglobulin II" fraction was estimated by difference. The conventional conversion factor 6.25 was applied to all fractions to calculate protein content from protein nitrogen values.

TABLE II

*Partition of serum proteins by the Howe method: hyperglobulinemia due to lymphogranuloma venereum, sarcoid, miscellaneous infections and cirrhosis*  
(Results expressed in grams per 100 cc. serum)

Number	Sex, age	Diagnosis	Total proteins	Albumin	Total globulins	Euglobulin	Pseudoglobulins		
							Total	I	II
1	F50	Lymphogranuloma venereum	11.2	3.5	7.7	3.4	4.3	3.8	0.5
2	F52	Lymphogranuloma venereum	9.8	3.7	6.1	2.2	3.9	3.3	0.6
3	F32	Lymphogranuloma venereum	9.2	3.5	5.7	2.7	3.0	2.2	0.8
4	M45	Lymphogranuloma venereum	9.2	3.3	5.9	1.8	4.1	3.4	0.7
5	F53	Lymphogranuloma venereum	9.1	2.4	6.7	3.0	3.7	3.3	0.4
6	M45	Lymphogranuloma venereum	9.0	2.7	6.3	2.0	4.3	3.7	0.6
7	M34	Lymphogranuloma venereum	8.1	4.7	3.4	0.5	2.9	2.2	0.7
8	M39	Lymphogranuloma venereum	8.0	3.2	4.8	1.0	3.8	3.2	0.6
9	F48	Lymphogranuloma venereum	7.9	3.5	4.4	1.0	3.4	2.6	0.8
10	M59	Lymphogranuloma venereum	7.2	3.7	3.5	1.1	2.4	2.1	0.3
11	M48	Sarcoid	9.7	3.9	5.8	2.3	3.5	3.1	0.4
12	F29	Sarcoid	9.6	4.2	5.4	2.1	3.3	2.8	0.5
13	F58	Sarcoid	8.3	2.0	6.3	3.1	3.2	2.8	0.4
14	F24	Sarcoid	7.5	3.8	3.7	0.9	2.8	2.3	0.5
15	F53	Sarcoid	7.0	1.7	5.3	1.8	3.5	3.2	0.3
16	M26	Poikilodermatomyositis	9.4	4.4	5.0	1.9	3.1	2.5	0.6
17	F38	Undiagnosed	8.9	4.2	4.7	1.4	3.3	2.5	0.8
18	F17	Lupus erythematosus disseminatus	8.3	3.0	5.3	1.9	3.4	2.4	1.0
19	M51	Hemolytic streptococcus bacteremia	8.3	4.1	4.2	0.9	3.3	2.7	0.6
20	F28	Hemolytic streptococcus bacteremia	8.0	4.3	3.7	0.9	2.8	2.2	0.6
21	F23	Lupus erythematosus disseminatus	7.9	2.8	5.1	1.4	3.7	3.3	0.4
22	F21	Undiagnosed	7.9	3.8	4.1	0.8	3.3	2.7	0.6
23	F22	Tuberculous lymphadenitis	7.9	4.7	3.2	0.5	2.7	2.1	0.6
24	F45	Leprosy	7.6	3.4	4.2	0.9	3.3	2.7	0.6
25	M37	Subacute bacterial endocarditis	7.3	3.1	4.2	1.0	3.2	2.7	0.5
26	M38	Leukemia	7.2	3.4	3.8	0.7	3.1	2.5	0.6
27	F16	Rheumatic fever, nephritis	7.2	3.8	3.4	0.6	2.8	2.1	0.7
28	M53	Rheumatoid arthritis	7.1	3.5	3.6	0.9	2.7	2.0	0.7
29	M28	Pylephlebitis	7.0	3.4	3.6	0.8	2.8	2.1	0.7
30	F55	Undiagnosed	6.9	2.7	4.2	0.8	3.4	2.8	0.6
31	F35	Cirrhosis	7.7	3.4	4.3	0.8	3.5	3.0	0.5
32	F51	Cirrhosis	7.5	2.4	5.1	1.1	4.0	3.7	0.3
33	M48	Cirrhosis	7.3	2.9	4.4	1.2	3.2	2.7	0.5
34	M48	Cirrhosis	7.1	2.9	4.2	1.1	3.1	2.7	0.4
35	M49	Cirrhosis	6.9	3.4	3.5	0.9	2.6	2.2	0.4
36	F67	Cirrhosis	6.6	2.1	4.5	1.1	3.4	2.9	0.5
37	M59	Cirrhosis	6.5	2.4	4.1	0.7	3.4	2.8	0.6
38	M52	Cirrhosis	6.4	1.6	4.8	1.6	3.2	3.0	0.2
39	M68	Cirrhosis	5.7	2.0	3.7	0.9	2.8	2.4	0.4
40	M45	Cirrhosis	5.1	2.1	3.0	0.5	2.5	2.2	0.3

tions tend to be lower in the A than in the B series but the difference is small in normal sera and becomes imperceptible in conditions causing hyperglobulinemia.<sup>1</sup>

Due to overlapping of precipitation limits, incomplete precipitation, adsorption, and other causes (20, 21), the separation of fractions by salting-out procedures such as Howe's method is

<sup>1</sup> The terms "euglobulin," "pseudoglobulin I" and "pseudoglobulin II" will be used throughout in the sense employed by Howe, to designate arbitrarily defined serum protein mixtures precipitable, respectively, in 30 volumes of (approximately) 1.0, 1.25 and 1.5 M sodium sulfate solution. Each of these fractions contains both water-insoluble and water-soluble serum globulins (19), the "euglobulins" and "pseudoglobulins" of classical usage.

very imperfect, particularly with respect to the globulin subfractions. Moreover, the composition of fractions precipitable with the same concentration of salt may be quite different in different conditions. For example, the large "euglobulin" fraction in some cases of multiple myeloma doubtless contains proteins not present in the corresponding "euglobulin" fraction of normal serum and may be different in composition from the "euglobulin" fraction in infections.

#### *Serum protein partitions in chronic infections presenting hyperproteinemia, and in cirrhosis*

The diseases in which marked hyperglobulinemia commonly occurs, and which are there-

fore suitable for study of the serum proteins in hyperproteinemia, fall into three main groups: certain chronic infections, cirrhosis and multiple myeloma. Data representative of results with the Howe method in chronic infections and in cirrhosis are summarized in Table II. Multiple myeloma, a special case, will be considered separately.

1. *Total serum proteins.* The disease most frequently represented in our series is lymphogranuloma venereum which, as indicated elsewhere (22, 23), is a common cause of marked hyperproteinemia in this area, as in many other localities (24, 25, 26, and others). Apart from multiple myeloma, all our total protein values of 10.0 grams per cent or over (as high as 11.7 grams per cent) occurred in lymphogranuloma venereum, with the exception of one case of kala-azar (11.4 grams per cent), one of sarcoid (10.0 grams per cent) and one of undetermined cause (10.0 grams per cent). Less marked and less consistent hyperproteinemia was observed in a variety of other conditions, as indicated in Table II.

2. *Serum albumins.* The significant features of the relation of serum albumin levels to total serum proteins, in diseases presenting hyperglobulinemia, are brought out in Figure 1. This figure includes 90 points representing results in 80 cases of lymphogranuloma venereum, 85 points in 70 patients with other infections, 70 points in 62 cases of cirrhosis and the 36 normal values of Table I A. There is clearly no correlation, positive or negative, between the albumin and the total protein content of the serum in these conditions. Serum albumin levels higher than those observed in normal subjects were not encountered (except in states of marked hemoconcentration); when the albumins are affected, as they are in a variety of debilitating conditions (27), the trend uniformly is downward irrespective of the total protein level. In cirrhosis the serum albumin content often is disproportionately low in relation to the total protein content. All these findings conform with the general experience.

3. *Serum globulins.* Data from the same cases presenting hyperglobulinemia are plotted in the lower part of Figure 1 to illustrate the well-recognized relation of serum globulins to total

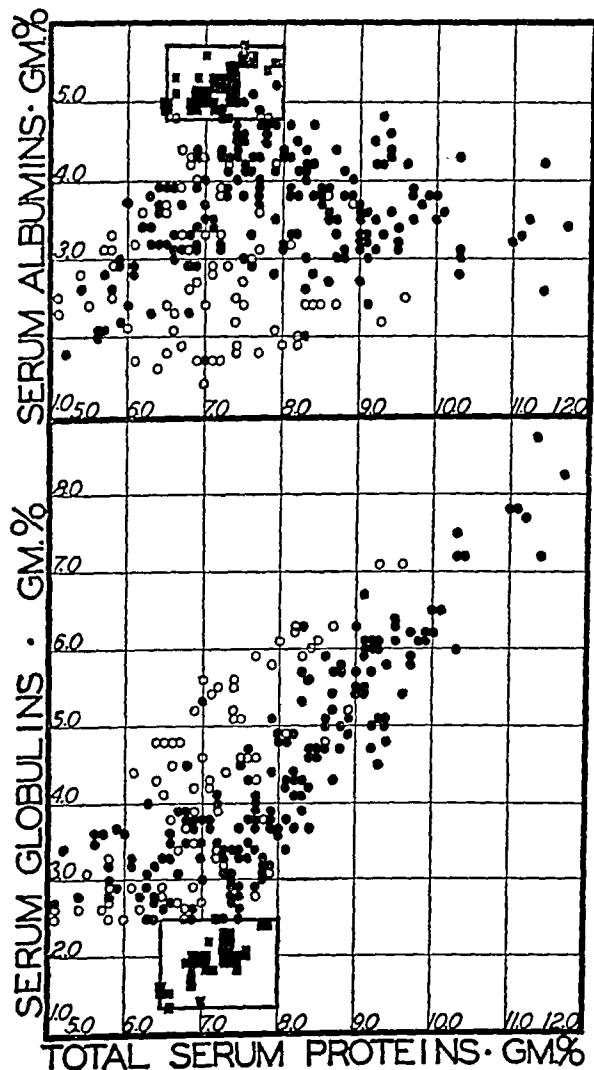


FIG. 1. RELATION OF ALBUMIN CONTENT (UPPER) AND OF GLOBULIN CONTENT (LOWER) TO TOTAL PROTEIN CONTENT OF SERUM IN DISEASES PRESENTING HYPERGLOBULINEMIA

The solid dots represent cases of lymphogranuloma venereum and other infections; hollow dots represent cases of cirrhosis; squares are normal values, normal range enclosed by heavy lines.

protein levels. The points show a definite trend with a sharp, positive slope which reflects the direct proportionality between the total globulin and total protein content of the serum. The aberrant position of many points representing cases of cirrhosis is indicative of their disproportionately high globulin content. These points also show how frequently marked hyperglobulinemia, if accompanied by sufficiently de-

TABLE II

*Partition of serum proteins by the Howe method: hyperglobulinemia due to lymphogranuloma venereum, sarcoid, miscellaneous infections and cirrhosis*  
(Results expressed in grams per 100 cc. serum)

Number	Sex, age	Diagnosis	Total proteins	Albumin	Total globulins	Euglobulin	Pseudoglobulins	
							Total	I
1	F50	Lymphogranuloma venereum	11.2	3.5	7.7	3.4	4.3	3.8
2	F52	Lymphogranuloma venereum	9.8	3.7	6.1	2.2	3.9	3.3
3	F32	Lymphogranuloma venereum	9.2	3.5	5.7	2.7	3.0	2.2
4	M45	Lymphogranuloma venereum	9.2	3.3	5.9	1.8	4.1	3.4
5	F53	Lymphogranuloma venereum	9.1	2.4	6.7	3.0	3.7	3.3
6	M45	Lymphogranuloma venereum	9.0	2.7	6.3	2.0	4.3	3.7
7	M34	Lymphogranuloma venereum	8.1	4.7	3.4	0.5	2.9	2.2
8	M39	Lymphogranuloma venereum	8.0	3.2	4.8	1.0	3.8	3.2
9	F48	Lymphogranuloma venereum	7.9	3.5	4.4	1.0	3.4	2.6
10	M59	Lymphogranuloma venereum	7.2	3.7	3.5	1.1	2.4	2.1
11	M48	Sarcoid	9.7	3.9	5.8	2.3	3.5	3.1
12	F29	Sarcoid	9.6	4.2	5.4	2.1	3.3	2.8
13	F58	Sarcoid	8.3	2.0	6.3	3.1	3.2	2.8
14	F24	Sarcoid	7.5	3.8	3.7	0.9	2.8	2.3
15	F53	Sarcoid	7.0	1.7	5.3	1.8	3.5	3.2
16	M26	Poikilodermatomyositis	9.4	4.4	5.0	1.9	3.1	2.5
17	F38	Undiagnosed	8.9	4.2	4.7	1.4	3.3	2.5
18	F17	Lupus erythematosus disseminatus	8.3	3.0	5.3	1.9	3.4	2.4
19	M51	Hemolytic streptococcus bacteremia	8.3	4.1	4.2	0.9	3.3	2.7
20	F28	Hemolytic streptococcus bacteremia	8.0	4.3	3.7	0.9	2.8	2.2
21	F23	Lupus erythematosus disseminatus	7.9	2.8	5.1	1.4	3.7	3.3
22	F21	Undiagnosed	7.9	3.8	4.1	0.8	3.3	2.7
23	F22	Tuberculous lymphadenitis	7.9	4.7	3.2	0.5	2.7	2.1
24	F45	Leprosy	7.6	3.4	4.2	0.9	3.3	2.7
25	M37	Subacute bacterial endocarditis	7.3	3.1	4.2	1.0	3.2	2.7
26	M38	Leukemia	7.2	3.4	3.8	0.7	3.1	2.5
27	F16	Rheumatic fever, nephritis	7.2	3.8	3.4	0.6	2.8	2.1
28	M53	Rheumatoid arthritis	7.1	3.5	3.6	0.9	2.7	2.0
29	M28	Pylephlebitis	7.0	3.4	3.6	0.8	2.8	2.1
30	F55	Undiagnosed	6.9	2.7	4.2	0.8	3.4	2.8
31	F35	Cirrhosis	7.7	3.4	4.3	0.8	3.5	3.0
32	F51	Cirrhosis	7.5	2.4	5.1	1.1	4.0	3.7
33	M48	Cirrhosis	7.3	2.9	4.4	1.2	3.2	2.7
34	M48	Cirrhosis	7.1	2.9	4.2	1.1	3.1	2.7
35	M49	Cirrhosis	6.9	3.4	3.5	0.9	2.6	2.2
36	F67	Cirrhosis	6.6	2.1	4.5	1.1	3.4	2.9
37	M59	Cirrhosis	6.5	2.4	4.1	0.7	3.4	2.8
38	M52	Cirrhosis	6.4	1.6	4.8	1.6	3.2	3.0
39	M68	Cirrhosis	5.7	2.0	3.7	0.9	2.8	2.4
40	M45	Cirrhosis	5.1	2.1	3.0	0.5	2.5	2.2

tions tend to be lower in the A than in the B series but the difference is small in normal sera and becomes imperceptible in conditions causing hyperglobulinemia.<sup>1</sup>

Due to overlapping of precipitation limits, incomplete precipitation, adsorption, and other causes (20, 21), the separation of fractions by salting-out procedures such as Howe's method is

<sup>1</sup> The terms "euglobulin," "pseudoglobulin I" and "pseudoglobulin II" will be used throughout in the sense employed by Howe, to designate arbitrarily defined serum protein mixtures precipitable, respectively, in 30 volumes of (approximately) 1.0, 1.25 and 1.5 M sodium sulfate solution. Each of these fractions contains both water-insoluble and water-soluble serum globulins (19), the "euglobulins" and "pseudoglobulins" of classical usage.

very imperfect, particularly with respect to globulin subfractions. Moreover, the concentration of fractions precipitable with the same concentration of salt may be quite different under different conditions. For example, the "euglobulin" fraction in some cases of multiple myeloma doubtless contains proteins not in the corresponding "euglobulin" fraction of normal serum and may be different in composition from the "euglobulin" fraction in infectious

#### *Serum protein partitions in chronic infectious hyperproteinemia, and in cirrhosis*

The diseases in which marked hyperglobulinemia commonly occurs, and which a

TABLE III  
*Partition of serum proteins by the Howe method: multiple myeloma*  
 (Results expressed in grams per 100 cc. serum)

Number	Sex, age	Date	Total protein	Albumin	Total globulins	Euglobulin	Pseudo-globulins			Bence-Jones protein in urine	Basis for diagnosis
							Total	I	II		
A. Cases with hyperproteinemia											
1. HYPERGLOBULINEMIA WITH HYPEREUGLOBULINEMIA											
1.	M50	August 12, 1941 August 14, 1941	13.6 13.7	3.6 3.4	10.0 10.3	7.2 7.1	2.8 3.2	2.0	0.8	++++	Bone pains, marked macrocytic, normochromic anemia. X-rays: generalized decalcification, collapsed vertebrae. Autopsy August 22, 1941.
2.	M46	November 30, 1940	12.2	1.8	10.4	9.4	1.0	0.7	0.3	++++	Tumor of skull, hemorrhages, marked hyperchromic anemia, bone pain. X-rays: osteolytic area calvarium, generalized decalcification. Autopsy December 7, 1940.
3.	M63	April 18, 1939 April 25, 1939 May 4, 1939	10.6 12.0 9.6	2.6 2.6 2.3	8.0 9.4 7.3	2.1 4.5 3.9	5.9 4.9 3.4	5.2 4.2 2.7	0.7 0.7 0.7	++	Pain in back, chest; dyspnea, uremia. X-rays: osteolytic areas ribs, skull, spine. Autopsy May 6, 1939.
4.	M39	February 1, 1935 February 13, 1935 April 23, 1935 February 16, 1936	10.0 11.0 10.3 9.2	3.2 3.5 4.2 3.1	6.8 7.5 6.1 6.1	4.1 4.6 3.6 2.3	2.7 2.9 2.5 3.8	1.9 2.0	0.8 0.9	0 ++++	Chest pains, weakness, fever, marked hyperchromic anemia. X-rays: skeleton essentially negative. Autopsy February 18, 1936.
5.	F57	August 25, 1936 September 11, 1936 September 29, 1936	9.2 8.9 9.6	2.6 2.5 2.4	6.6 6.4 7.2	4.7 4.4 5.5	1.9 2.0 1.7	1.2	0.7	0	Weight loss, pain in chest, dyspnea, marked anemia. X-rays: skeleton negative. Autopsy October 19, 1936.
6.	M56	October 18, 1940	9.5	3.2	6.3	4.2	2.1	1.8	0.3	0	Bone pains, anemia. X-rays: generalized decalcification, collapsed vertebrae, punched-out areas femora. Autopsy October 25, 1940. Case of Dr. J. Tullis.
7.	M58	June 11, 1940 October 3, 1940 October 10, 1940	8.1 10.6 11.1	3.6 2.7 2.5	4.5 7.9 8.6	0.6 2.6 3.2	3.9 5.3 5.4			0	Pain in spine, ribs; weight loss, anemia. X-rays: multiple osteolytic areas ribs, spine. Hypercalcemia. No primary visceral tumor found. Case of Dr. I. Woodruff.
8.	F58	October 21, 1939 November 4, 1939	9.5 9.0	3.5 4.3	6.0 4.7	2.9 2.4	3.1 2.3			0	Moderate weakness, bone pain. X-rays: extensive punched-out areas in bones. No primary visceral tumor found. Case of Dr. L. Cotter.
9.	M58	May 11, 1936	8.6	3.8	4.8	1.8	3.0			+++	No clinical data. X-rays: multiple punched-out areas in bones. Biopsy May, 1936. Case of Dr. V. Brown.
2. HYPERGLOBULINEMIA WITHOUT HYPEREUGLOBULINEMIA											
10.	M64	November 5, 1938 December 6, 1938 January 5, 1939 January 9, 1939 May 15, 1939 November 10, 1939 June 19, 1940 July 9, 1940	9.4 9.4 9.0 8.9 9.9 9.8 9.9 10.2	2.9 2.7 2.0 2.4 2.5 2.5 2.2 2.7	6.5 6.7 6.6 6.5 7.4 7.3 7.7 7.5	<0.1 <0.1 <0.1 <0.1 <0.1 0.3 <0.1 <0.1	6.5 6.7 6.6 6.5 7.4 7.0 7.7 7.5			0	Weakness, gastro-intestinal bleeding, marked hyperchromic anemia. X-rays: slight generalized decalcification. Autopsy July 10, 1940.
11.	M63	June 27, 1940 July 1, 1940 July 23, 1940 October 15, 1940	8.2 8.1 8.4 7.6	6.2* 6.0 6.7 5.5	2.0* 2.1 1.7 2.1	<0.1 0.1 <0.1 <0.1	2.0 2.0 1.7 2.1			++++	Weakness, chest pain, dyspnea, tumor of chest wall, uremia. X-rays: extensive osteolytic areas skeleton. Autopsy October 27, 1940.
12.	M70	July 31, 1936 August 4, 1936	10.9 11.1	2.5 2.3	8.4 8.8	0.3 0.4	8.1 8.4	7.5	0.6	+++	Bone pains chest, back; weight loss; tumors of skull. X-rays: many punched-out areas ribs, spine, pelvis. Hypercalcemia. Plasma cells in blood smear.
13.	F56	April 13, 1939 April 20, 1939	9.1 8.3	4.0 3.7	5.1 4.6	0.8 0.4	4.3 4.2	3.6 3.6	0.7 0.6	0	Bone pains back, chest; weight loss, anemia. X-rays: osteolytic areas ribs with large soft tissue mass attached. Case of Dr. I. Woodruff.
14.	F43	May 26, 1936 May 28, 1936 July 8, 1936	8.0 8.0 6.4	3.5 3.4 3.2	4.5 4.6 3.2	0.5 0.7 0.6	4.0 3.9 2.6			0	Autopsy July 23, 1936. For details see Holman (40).
15.	M68	October 24, 1938	9.4	3.8	5.6	<0.1	5.6				Bone pains. X-rays: numerous punched-out areas in many skeletal parts; pathological fractures. Case of Dr. J. Olpp.
3. HYPERPROTEINEMIA, GLOBULIN SUBFRACTIONS NOT DETERMINED											
16.	M48	February 28, 1930 May 15, 1930	12.1 12.2	2.9 2.8	9.2 9.4					0	Weight loss, weakness, dyspnea, marked normochromic anemia. X-rays: skeleton negative. Autopsy April 8, 1931.†
17.	F64	December 5, 1935	11.5	1.9	9.6					0	Bone pains back, legs; moderate anemia. X-rays: large destructive lesion left ilium, generalized decalcification. Biopsy November 26, 1935.
18.	M59	October 25, 1933 October 29, 1933	10.1 9.9	2.9	7.0					±	For clinical and x-ray evidence, see Gutman, Tyson, Gutman (41), Table III, Case 4.



TABLE III—Continued

Number	Sex, age	Date	Total proteins	Albumin	Total globulins	Euglobulin	Pseudoglobulins			Bence-Jones protein in urine	Basis for diagnosis
							Total	I	II		
3. HYPERPROTEINEMIA, GLOBULIN SUBFRACTIONS NOT DETERMINED—Continued											
19.	M64	February 6, 1934	9.0							+	Autopsy February 26, 1934. For details see Gutman, Tyson, Gutman (41), Table III, Case 1.
20.	F56	April 20, 1933	8.3								For clinical and x-ray evidence see Gutman, Tyson, Gutman (41), Table III, Case 5.
B. Cases with total serum proteins <8.0 grams per cent											
4. NORMAL TOTAL SERUM PROTEIN LEVELS WITH HYPERGLOBULINEMIA											
21.	F62	April 11, 1939	7.1	3.3	3.8	0.2	3.6			0	Pain chest, weight loss, tumors of skull, hyperchromic anemia. X-rays: many punched-out areas in bones, fractures. Biopsy April 18, 1939.
		April 17, 1939	7.4	3.6	3.8	<0.1	3.8	3.1	0.7		
		April 25, 1939	7.9	3.8	4.1	0.1	4.0	3.4	0.6		
22.	M65	March 10, 1941	7.8	2.6	5.2	0.2	5.0			0	Weight loss, weakness. X-rays: osteolytic areas femora, spine, ribs. Biopsy April 3, 1941. Case of Dr. I. Woodruff.
		March 13, 1941	7.5	2.5	5.0	0.2	4.8	4.0	0.8		
23.	M50	December 17, 1937	7.0	3.0	4.0	0.1	3.9			0	Pain chest, back; paraplegia. X-rays: osteolytic areas spine, ribs; collapsed vertebra. Autopsy January 7, 1938.
		December 21, 1937	7.1	2.8	4.3	0.2	4.1	3.2	0.9		
		December 23, 1937	7.3	2.8	4.5	0.1	4.4				
		January 1, 1938	6.9	2.5	4.4	0.2	4.2				
24.	F60	November 12, 1936	7.4	2.8	4.6	0.9	3.7			0	Bone pains, weight loss, weakness, fractures, tumors of scalp. X-rays: many punched-out areas in bones. Autopsy March 7, 1937.
		November 23, 1936	7.4	2.9	4.5	1.2	3.3				
		December 9, 1936	7.0	2.9	4.1	0.7	3.4				
		January 27, 1937	7.8	3.3	4.5	0.8	3.7				
5. APPARENTLY NORMAL DISTRIBUTION OF SERUM PROTEIN FRACTIONS											
25.	F58	April 28, 1939	6.6	4.5	2.1	0.1	2.0	1.2	0.8	++	Pains in chest. X-rays: multiple punched-out areas in bones. Biopsy May 26, 1939.
		June 2, 1939	6.7	4.8	1.9	<0.1	1.9	1.2	0.7		
26.	M57	January 5, 1938	5.7	3.9	1.8	0.4	1.4	0.6	0.8	++++	Weight loss, weakness, fainting spells. X-rays: extensive osteolytic areas of bones. Biopsy January 21, 1938.
		January 25, 1938	5.6	4.0	1.6	<0.1	1.6				
27.	M59	November 19, 1940	6.4	4.8	1.6	<0.1	1.6			+++	Bone pains, weight loss, weakness, marked anemia. X-rays: many osteolytic areas in bones. Biopsy October, 1940. Case of Dr. O. Province.
28.	M41	May 16, 1941	7.1	5.3	1.8					0	Spontaneous fracture left femur. X-rays: solitary (?) plasmacytoma. Biopsy June 20, 1941.
		July 7, 1941	6.9	4.8	2.1	0.3	1.8	1.0	0.8		
29.	M61	November 8, 1935	5.5	4.1	1.4					++++	Autopsy February 27, 1936. For details see Fowler (42).
		November 20, 1935	5.8	3.3	2.5						
		December 5, 1935	5.8	3.8	2.0						
		February 17, 1936	5.5	3.6	1.9	0.2	1.7				
30.	M68	November 15, 1939	6.1	4.0	2.1	0.3	1.8			0	Compression fracture vertebra with paraplegia. X-rays: multiple punched-out areas in bones. Biopsy November 16, 1939.
31.	M55	March 13, 1939	7.2	4.9	2.3	0.1	2.2				Bone pains back, fracture. X-rays: osteolytic areas spine, femur, with collapsed vertebra, fractured femur. Biopsy 1940.
32.	F49	April 29, 1938	6.8	4.8	2.0	0.4	1.6			+++	Purpura, marked hyperchromic anemia. X-rays: extensive punched-out areas in bones. No primary visceral tumor found.
		May 3, 1938	7.7	5.6	2.1	0.2	1.9				
33.	M40	April 22, 1936	7.7	5.4	2.3	0.4	1.9			+++	Weakness, marked anemia, hemorrhages. X-rays: many osteolytic areas in bones.
34.	F55	July 27, 1938	7.0	4.9	2.1	0.3	1.8			±	Large tumor skull, pain. X-rays: extensive osteolytic areas in bones with collapsed vertebra. No primary visceral tumor found.
35.	M52	July 13, 1939	7.0	5.0	2.0					0	Bone pains back, chest; weight loss, dyspnea. X-rays: extensive punched-out areas bones. Autopsy July 17, 1939.
6. NORMAL TOTAL SERUM PROTEIN LEVELS, NOT FRACTIONATED											
36.	M47	February 21, 1934	6.3							0	For data see Gutman, Tyson, Gutman (41). Table III, Case 6.
		March 1, 1934	6.9								
37.	M60	October 24, 1932	6.1							+	For data see Gutman, Tyson, Gutman (41). Table III, Case 3.
7. MARKED HYPOPROTEINEMIA											
38.	F58	May 8, 1934	4.0							++++	Autopsy July 5, 1934. For details see Gutman, Tyson, Gutman (41). Table III, Case 2.
		May 17, 1934	3.5								

\* For reasons stated in the text, this case is included in the group with hyperglobulinemia.

† This case was presented elsewhere [(30); see Table IV, Case J. P.] as lymphosarcoma, a diagnosis made at autopsy performed at another hospital. The sections were reviewed by Dr. A. M. Pappenheimer and others who identified the tumor cells as myeloma (plasma) cells.

All of the  $\gamma$ , about three-fourths of the  $\beta$  and one-fourth of the  $\alpha$  components were removed by precipitation in 21.5 per cent sodium sulfate. As indicated, the Howe albumin filtrate also contains appreciable amounts of  $\alpha$  and  $\beta$  components, presumably Hewitt's globoglycoid (37). The results in normal human serum correspond with those in normal horse serum fractionated with ammonium sulfate (1, 35, 37).

#### *Serum protein partitions in multiple myeloma*

Table III summarizes our data with the Howe method in 38 cases of multiple myeloma. The diagnosis was established by autopsy or bone marrow biopsy in 26 patients; in 12 instances opportunity for histological examination was lacking and the diagnosis rests upon clinical, roentgenological and chemical evidence, as indicated in Table III. Except where specific acknowledgments are made, the cases are from the records of the Presbyterian Hospital for the years 1930 to 1941.

It is obvious upon inspection of the data in Table III that multiple myeloma represents a special case in the distribution of serum protein fractions in hyperproteinemia, with anomalies that are without precedent in our experience with any other disease. In one instance (Case 11) hyperproteinemia appeared to be due to hyperalbuminemia; in another (Case 10) partly to a distinct increase in "pseudoglobulin II." In several instances, marked hyperglobulinemia appeared to result solely from increased "pseudoglobulin I" without any accompanying rise in "euglobulin." These peculiarities correspond with the experience of others who have found that hyperproteinemia in multiple myeloma may be due chiefly to increases in any of the following fractions: euglobulin (5, 6, 43, and others), pseudoglobulin I (11, 31, 32, and others), pseudoglobulin II (10, 32, and others), albumin (44, 45, and others), fibrinogen (43), Bence-Jones protein (46, and others), unclassified abnormal proteins (47, 48).

#### *A. Howe fractionation*

1. *Total serum protein levels.* Twenty patients presented protein levels of 8 grams per cent or more (Table III A), 18 did not have associated

hyperproteinemia (Table III B). The values of 12.0 to 13.7 grams per cent encountered in Cases 1, 2, 3 and 16 are the highest in our records. They are considerably lower, however, than have been reported in this disease (49, 50, and others). The incidence of hyperproteinemia in our series, 53 per cent, is less than that of most estimates from published sources: 43 per cent (50); 57 per cent (33); 69 per cent (45); 175 of 282 cases or 62 per cent, according to our latest tally. But, owing to recent emphasis upon hyperproteinemia and related phenomena, the literature perhaps includes a disproportionate number of cases with elevated serum protein values.

Cases 26, 29 and 38 had total serum protein levels less than 6 grams per cent. The marked hypoproteinemia (3.5 grams per cent) observed in Case 38 corresponds with observations by Chester (51) and others.

2. *Serum albumins.* The serum albumin fraction in multiple myeloma, as in other wasting diseases, is usually decreased. The incidence of marked hypoalbuminemia seems to be unusually high: 12 of our cases presented levels less than 3.0 grams per cent, 9 of which on at least one occasion were 2.5 grams per cent or less. This proportion of marked hypoalbuminemia exceeds that in most infections or neoplasia and approaches the incidence in Laënnec's cirrhosis. No distinct correlation could be made out between the degree of proteinuria and the serum albumin content.

The apparent hyperalbuminemia of Patient 11, who was not dehydrated, was investigated further with the following results: (a) High "albumin" values were not obtained if the serum-22.2 per cent sodium sulfate mixture remained 48 hours at 37° before filtration. A sample repeatedly yielding 6.0 grams per cent filtrate protein after 3 hours' precipitation gave only 4.4 grams per cent filtrate protein if filtration was carried out after 48 hours' precipitation. A subsequent sample containing 6.7 grams per cent filtrate protein, if 3 or 24 hours were allowed for precipitation, gave only 4.5 grams per cent protein in filtrates obtained after 48 hours. (b) Precipitation in 21.5 per cent sodium sulfate buffered to pH 6.8 yielded 3.7 grams per cent filtrate protein, as compared with 6.0 grams per cent obtained with unbuffered solutions. (c) A sample of

serum yielding 5.5 grams per cent protein in 21.5 per cent sodium sulfate filtrates gave only 4.7 grams per cent filtrate protein after precipitation with 23.0 per cent sodium sulfate. (In our experience, these variations in the salting-out technique ordinarily cause only insignificant changes in the results of serum albumin determinations.) Electrophoretic fractionation showed 4.1 grams per cent albumin in the serum sample which by Howe's method contained 6.0 grams per cent filtrate protein after 3 hours' precipitation and 4.4 grams per cent after 48 hours. The precipitin technique, applied to a sample of this serum by Dr. F. E. Kendall, also indicated an albumin content of 4.1 grams per cent. Evidently, the serum in Case 11 contained about 2 grams per cent of a protein component which was not serum albumin but nevertheless remained in solution in 21.5 per cent sodium sulfate unless precipitation were allowed to continue much longer than prescribed by Howe.<sup>4</sup>

If such a protein component were present in lesser concentration, or superimposed upon a lower albumin content, the total "albumin" level might fall within normal limits and so escape attention. Several of the 11 cases in our series, with apparently normal distribution of Howe fractions (Table III B), presented "albumin" levels within normal limits but unexpectedly robust for patients who were distinctly wasted but not markedly dehydrated. Those with highest "albumin" levels (Case 32: 5.6 grams per cent; Case 33: 5.4 grams per cent; Case 35: 5.0 grams per cent) unfortunately were not studied further. But in Case 27, it could be shown electrophoretically that, of 4.8 grams per cent albumin indicated by Howe's method, only 3.2 grams per cent migrated with the mobility of serum albumin; and that an abnormal protein component was present, forming an extra boundary between the  $\beta$  and  $\gamma$  peaks. In Cases 25 and 28 similar, though less marked, differences were observed between salting-out and electrophoretic values for albumin. The conclusion seems warranted that, unless special precautions are taken, the Howe method (which gives high

values for albumin in normal serum) occasionally gives much too high values for serum albumins in multiple myeloma; the error being due to an abnormal protein component, not albumin, in the 21.5 per cent sodium sulfate filtrate of some myelomatous sera. The error may be obvious because the "albumin" values become improbably high; more often perhaps the "albumin" levels do not exceed the normal maximum and escape correction.

3. *Serum globulins.* Total globulin levels over 3.0 grams per cent were found in 24 cases, inclusive of Cases 11, 19 and 20; in the 2 latter instances, the proteins were not fractionated but the increased total content implies an increase in globulins. The levels of 9.0 to 10.4 grams per cent globulin in Cases 1, 2, 3, 16 and 17, though considerably lower than have been recorded in multiple myeloma, are the highest we have ever encountered in any disease. The incidence of hyperglobulinemia in our series (63 per cent) is greater than that of hyperproteinemia (53 per cent) because hyperglobulinemia in 4 cases was associated with lowering of the serum albumins sufficient to result in normal total protein levels.

In many cases of hyperglobulinemia due to multiple myeloma, the globulin increment was found to be composed of both "euglobulin" and "pseudoglobulin I," thus conforming with the characteristic pattern already described in chronic infections and in cirrhosis. This distribution of globulin subfractions was observed in our Cases 1 to 9 and 24. However, even in such apparently conventional myelomatous sera, the ratio "euglobulin"/total globulins is apt to be well outside the range of variation in this ratio observed in hyperglobulinemia due to other causes (Figure 3). The ratio may be excessively high because virtually all of the globulin increment is precipitable in 13.5 per cent sodium sulfate (Case 2); or unusually low (Cases 3 and 7). Similar sera containing increased "euglobulin" and "pseudoglobulin I," but in atypical proportions, have been reported by others.

A peculiarity of some sera in multiple myeloma is that definite hyperglobulinemia may be associated with no increase whatever in components precipitable in 13.5 per cent sodium sulfate ("euglobulin"), as first reported by Gros (10). His case showed marked elevation in both "pseu-

<sup>4</sup> In Case 1, serum preserved with aseptic precautions in the refrigerator for about one week spontaneously changed to give lower albumin values by the usual Howe technique.

doglobulin II" and "pseudoglobulin I" fractions (chiefly the former) without any rise in "euglobulin." This is a rare variant, even in multiple myeloma, and our series includes but one such case of hyperglobulinemia due chiefly to increase in "pseudoglobulin II" (Case 10, observations in early 1939). It is more common to find that the globulin increment (in hyperglobulinemia without hypereuglobulinemia) is composed almost in its entirety of components precipitable in 17.4 per cent sodium sulfate; an increased "pseudoglobulin I" fraction being the sole cause of hyperglobulinemia. Case 12 appears to have been the first recorded example of this anomaly (11), of which a number of instances have been described (31, 32, and others). In fact, this type of fractional distribution is of such high incidence (9 times in our present series, Cases 10 to 15, 21 to 23) and is so characteristic of multiple myeloma that it has definite diagnostic value.<sup>5</sup>

4. *Plasma fibrinogen.* Determinations were made only in Cases 4 (0.4 gram per cent), 10 (0.2 gram per cent), 12 (0.4 gram per cent), 23 (0.5, later increased to 1.0 gram per cent) and 24 (0.3 gram per cent). So far as can be judged from these values and from the clotting of the blood in the remaining cases, hyperproteinemia due principally to hyperinosis did not occur in our series.

### B. Electrophoretic fractionation

Longsworth, Shedlovsky and MacInnes (4) made the first electrophoretic studies in multiple myeloma (Cases 27, 28, 29 of their Table I; Cases 3, 21, 25, respectively, of this series, Tables III and IV). A marked increase in the  $\beta$  globulin peak was observed in 2 cases, due to the presence of components migrating with the mobility of  $\beta$  globulins; in one of these, the peak for fibrinogen also exceeded the limits for normal plasma. The third case yielded a normal pattern. Kekwick (13) reported results of electro-

phoretic and ultracentrifugal analyses of sera in 5 cases of multiple myeloma. Four of these showed a great increase in the  $\gamma$  globulin peak, associated with reduced percentage of albumin and essentially normal percentages of  $\alpha$  and  $\beta$  globulins. In the fifth case,  $\gamma$  globulin was present in less than normal amount but there was a marked increase in a component migrating with a mobility corresponding with that of  $\beta_2$  globulin.

Figure 5 and Table IV present electrophoretic data in sera of 7 additional cases of multiple myeloma. In Cases 1, 2 (Figure 5A) and 22 (Figure 5B) there was a very large increase in the  $\gamma$  peaks. The two latter sera also contained an extra component (designated  $M$ ), with a mobility between that of the  $\beta$  and  $\gamma$  components, corresponding with what in plasma would be the mobility of fibrinogen; the significance of this extra boundary will be considered later. The concentrations of albumin,  $\alpha$ ,  $\beta$ , and  $M$  were very low in the serum of Case 2.

In Cases 10 and 11 (Figures 5C and D), there was a marked increase in components moving with the mobility of  $\beta$  globulins. In Case 11, the mobility corresponded with that of the  $\beta_2$  fraction; the faster  $\beta_1$  component being indicated by a slight thickening on the forward side of the  $\beta$  peak. Neither of these sera showed any increase in  $\gamma$  globulins, nor was there any indication of an  $M$  boundary.

The serum of Case 27 (Figure 5E) gave an electrophoretic pattern simulating that of normal plasma: an extra component,  $M$ , was present. The serum of Case 28 (Figure 5F) yielded a normal pattern.

When the results of electrophoretic and Howe fractionations are compared (Table IV), the correspondence between the distribution of protein fractions by these two methods is found to be imperfect. In Cases 1 and 2 (Figure 5A), the extremely high "euglobulin" content is reflected in very large  $\gamma$  components; in Cases 10 (Figure 5C), 11 (Figure 5D), 21, 25, 27 (Figure 5E), 28 (Figure 5F), normal or low normal "euglobulin" contents corresponded with normal or low normal  $\gamma$  peaks.<sup>6</sup> However, an

<sup>5</sup> In spite of the extraordinary variation in serum protein patterns in multiple myeloma, it should be noted that the fractional distribution in any one patient did not change in type while under observation. In 10 of our cases, protein studies were made over periods from one month to more than 1½ years. In no instance was there any striking change in pattern; minor changes accompanied further progression of the disease in 3 cases.

<sup>6</sup> Dr. F. E. Kendall examined the sera in Cases 10 and 11 by the precipitin method and found correspondingly low "globulin I" figures: 0.3 and 0.5 gram per cent, respectively, the mean normal value being 0.8 gram per cent.

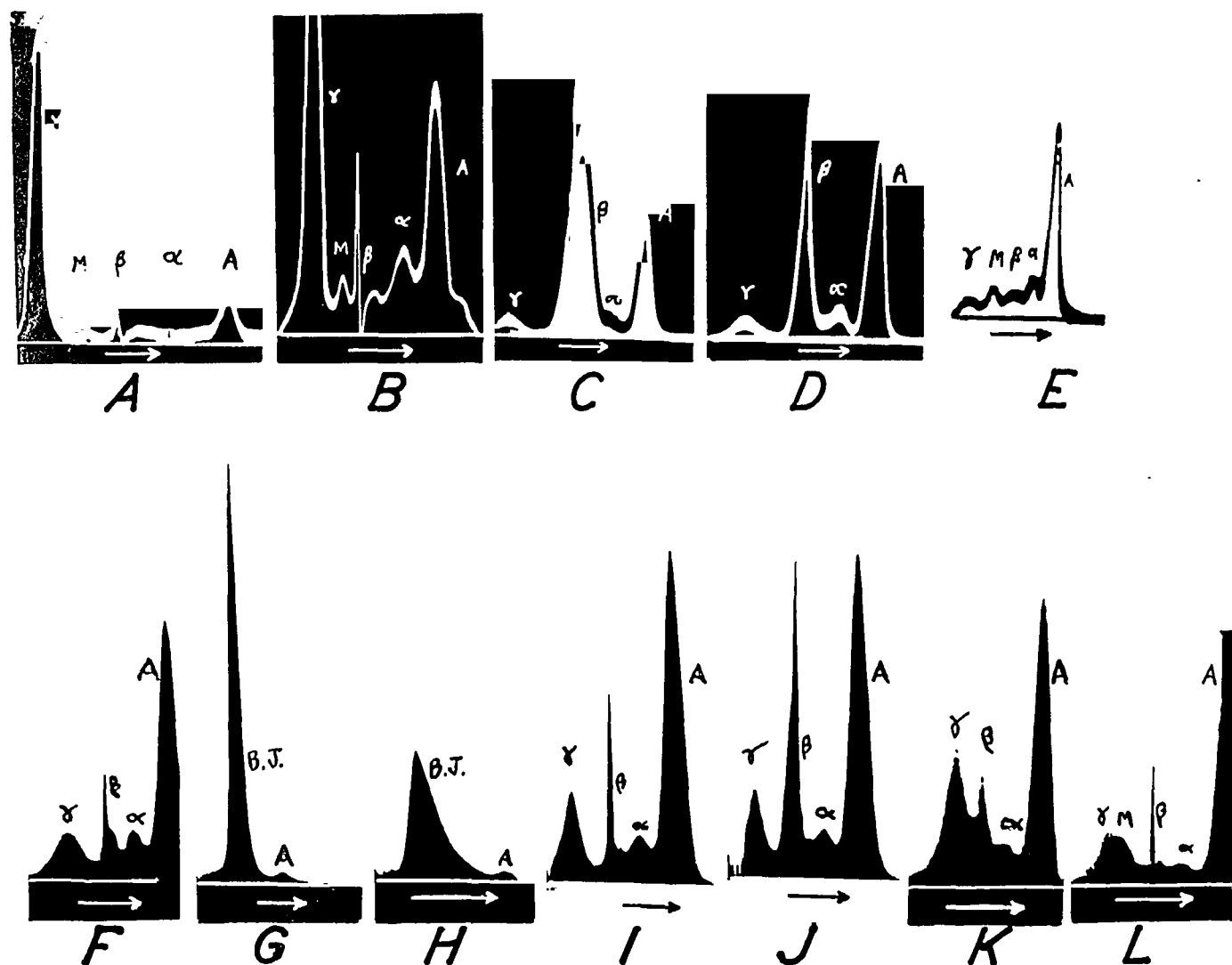


FIG. 5. ELECTROPHORETIC PATTERNS (DESCENDING LIMB) IN MULTIPLE MYELOMA

A through F represents results in serum of Cases 2 (A), 22 (B), 10 (C), 11 (D), 27 (E), 28 (F). Patterns in urine of Cases 11 (G) and 12 (H) show peaks for Bence-Jones protein and albumin. I is normal serum to samples of which were added Bence-Jones proteins from the urine of Cases 11 (J), 12 (K) and 3 (L). The patterns in the upper row were made with the Svensson cylindrical lens method.

extremely large  $\gamma$  component in Case 22 (Figure 5B) was associated with normal "euglobulin" values by Howe's method, in which the globulin increment appeared solely in the "pseudoglobulin I" fraction. In Case 3 (Figure 5b of reference 4), an increase in both "euglobulin" and "pseudoglobulin I" appeared as very large increases in the  $\beta$  and fibrinogen peaks.

In Cases 3, 10 and 21, large increases in "pseudoglobulin I" were reflected in large increases in components moving with the mobility of  $\beta$  globulins; in Cases 1, 2, 25, 27 and 28, normal or low normal "pseudoglobulin I" values were associated with normal or low normal  $\beta$  peaks. The discrepancy in this relation in Case 22 has been pointed out. In Case 11, a large  $\beta$  peak

was present, although no increase in the "pseudoglobulin I" fraction was apparent.

Large discrepancies in the albumin content of Cases 11 and 27, as determined by these two methods, have already been discussed. The presence of an extra component, M, in the electrophoretic analyses was in no instance revealed by the Howe method (for example, in the serum of Case 27, which appeared to be quite normal when examined by Howe's method). The plasma of Case 25 and the serum of Case 28 gave normal results by both methods.

### C. Bence-Jones proteinemia

Significant Bence-Jones proteinemia presumably occurs in some cases of multiple myeloma

TABLE IV

Comparison of results of fractionation of proteins by electrophoresis and Howe methods

Case	Diagnosis	Material	Electrophoretic fractionation													Howe fractionation (grams per cent)							
			Buffer, pH	Mobilities (U × 10 <sup>3</sup> )					Concentrations (per cent total area)					See figure	T.P.	Albumin	Globulin	Euglobulin	Pseudoglobulin			A/G	
				Λ	α	β	M	γ	Λ	α	β	M	γ						A/G	Total	I		II
	Normal	Serum	P 7.4	4.7	3.5	2.4		0.8	62.5	7.5	13.2		16.8	1.7	4	7.5	5.6	1.9	<0.1	1.9	1.0	0.9	2.9
2	L.V.	Serum	P 7.4	5.4	3.8	2.8		0.5	32.9	5.2	11.8		50.2	0.5	4	9.8	3.7	6.1	2.2	3.9	3.3	0.6	0.6
1	Myeloma	Serum	P 7.4	5.2	3.6	2.9		0.5	26.2	5.0	8.9		60.0			13.6	3.6	10.0	7.2	2.8	2.0	0.8	0.4
2	Myeloma	Serum	B 7.8	5.3	4.3	2.7	1.3	0.0	17.3	8.0	6.7	2.7	65.3	0.2	5A	12.2	1.8	10.4	9.4	1.0	0.7	0.3	0.2
3	Myeloma	Plasma	B 7.8	5.9	4.5	2.4		0.1	23.5	4.4	56.6	9.2	6.3	0.3		12.0	2.6	9.4	4.5	4.9	4.2	0.7	0.3
10	Myeloma	Serum	B 7.8	6.0	4.7	3.3		0.2	22.4	4.1	69.0		4.5	0.3	5C	10.2	2.7	7.5	<0.1	7.5	6.8	0.7	0.4
21	Myeloma	Plasma	B 7.8	5.5	4.2	2.7		0.2	47.0	3.2	38.0	3.2	8.5	0.9		7.9	3.8	4.1	0.1	4.0	3.4	0.6	0.9
22	Myeloma	Serum	B 7.8	6.5	5.0	3.2	2.3	0.7	31.0	10.5	9.8	3.6	45.1	0.5	5B	7.5	2.5	5.0	0.2	4.8	4.0	0.8	0.5
11	Myeloma	Serum	B 7.8	6.4	4.8	3.3		0.6	50.8	4.6	39.0		5.6	1.0	5D	8.1	6.0	2.1	0.1	2.0			2.9
25	Myeloma	Plasma	B 7.8	5.9	4.2	3.0	1.6	0.1	57.2	9.6	15.5	4.5	13.1	1.5		6.7	4.8	1.9	<0.1	1.9	1.2	0.7	2.5
27	Myeloma	Serum	B 7.8	6.5	5.1	3.9	2.5	0.7	50.2	12.3	9.5	13.2	14.8	1.0	5E	6.4	4.8	1.6	<0.1	1.6			3.0
28	Myeloma	Serum	P 7.4	5.0	3.5	2.3		0.7	58.1	9.8	14.1		18.0	1.4	5F	6.9	4.8	2.1	0.3	1.8	1.0	0.8	2.3
1	Myeloma	Urine	P 7.4	6.0			1.5		5.0			95.0											
3	Myeloma	Urine	P 7.4	6.5			2.0		24.0			76.0				0.33							
11	Myeloma	Urine	P 7.4	6.7			2.8		5.0			95.0			5G	0.91							
25	Myeloma	Urine	P 7.5				3.1		0			100.0				0.28							
29	Myeloma	Urine	P 7.4				2.5		0			100.0				1.41							
32	Myeloma	Urine	P 7.4	6.8			3.1		8.0			92.0				0.42							
12	Myeloma	Urine	P 7.4	5.4			1.8		8.0			92.0			5H	0.61							
Normal serum + Bence-Jones protein from urine of Case 11.....			P 7.4	4.8	3.5	2.2		0.8	49.2	6.8	29.9		13.9		5J	13.7	7.1	6.6	<0.1	6.6	0.9	5.7	
Normal serum + Bence-Jones protein from urine of Case 12.....			P 7.4	5.4	3.9	2.7		1.5	49.0	7.0	13.0		31.0		5K	9.6	6.9	2.7	<0.1	2.7	1.5	1.2	
Normal serum + Bence-Jones protein from urine of Case 3.....			P 7.4	5.1	3.5	2.6	1.5	1.0	54.0	7.0	13.0	11.0	15.0		5L								
Normal serum + Bence-Jones protein from urine of Case 32.....																9.0	5.8	3.2	0.7	2.5	1.4	1.1	

P = phosphate buffer, B = barbiturate buffer.

Figures for electrophoretic mobilities and concentrations in plasma of myeloma Cases 3, 21, 25 from Longworth, Shedlovsky and MacInnes (4). The concentration of M in these runs with plasma includes fibrinogen. All our concentrations are stated as per cent total refractive increment rather than in terms of albumin concentration because of the marked differences in albumin concentration in different cases of myeloma.

but, as no satisfactory method for quantitation of Bence-Jones proteins in serum is available, it is not known whether concentrations sufficient to cause hyperproteinemia develop and, if so, how frequently. Some of the older data indicating large accumulations in the blood are subject to the criticism (5, 6) that the methods employed do not discriminate adequately between Bence-Jones proteins and euglobulins. These criticisms appear not to apply to more recent estimations based on extraction (46, and others), protein solubility curves (52) and ultracentrifugation (53).

We have already described anomalous serum protein patterns encountered (in our experience) only in multiple myeloma. When the Howe method is used, these include occasional falsely high albumin values due to an abnormal protein in the 21.5 per cent sodium sulfate filtrate; hyperglobulinemia due wholly to an increase in

the fraction precipitable in 17.4 per cent sodium sulfate, or partly to an increase in the fraction precipitable in 21.5 per cent sodium sulfate. Electrophoretically, abnormal components have been described with mobilities between or overlapping the mobilities of the  $\beta$  and  $\gamma$  globulins. These peculiarities become significant when considered in connection with the salting-out and electrophoretic behavior of Bence-Jones protein in urine. They can be reproduced, in part, by adding Bence-Jones protein from urine to normal serum.

The precipitation limits of Bence-Jones proteins in urine vary widely in different cases of multiple myeloma. If ammonium sulfate is used, precipitation may begin anywhere between the concentration employed to throw down the more soluble euglobulins to that required to salt out albumins (54). However, in most cases precipitation begins at  $40 \pm 2$  per cent saturation

(the concentration used to salt out "pseudoglobulin I" from serum (55)) and ends at  $50 \pm 5$  per cent saturation ((54); personal observations). If sodium sulfate is used in urine not made strongly acid, the bulk of the Bence-Jones proteins usually remains in solution in 1.5 *M* filtrates; if such Bence-Jones proteins were present in serum and there showed the same solubility relations, they would appear largely in the albumin filtrates and give falsely high albumin values. In some urine samples (Cases 1, 3, 11, 32, 33, for example), partial precipitation of Bence-Jones proteins occurs in 21.5 per cent sodium sulfate, and in the urine of Cases 1, 3, 11 and 33, slight precipitation was observed also in 17.4 per cent but not in 13.5 per cent sodium sulfate solution; such proteins, if present in serum, presumably would appear in the "pseudoglobulin II" fraction or in both the "pseudoglobulin II" and "pseudoglobulin I" fractions. Correlations between the findings in serum and urine must necessarily be imperfect, however, if the concentrations in serum are sufficiently reduced by leakage through the kidneys.

Table IV gives the results of experiments in which urinary Bence-Jones proteins were added to normal serum of the composition indicated in that table. Of the Bence-Jones protein from the urine of Case 11 added to normal serum in an amount equivalent to 6.2 grams per cent, one-fourth appeared in the Howe albumin fraction, three-fourths precipitated out with the globulins; the latter coming down entirely in the "pseudoglobulin II" fraction. Of the Bence-Jones protein from the urine of Case 12 added to normal serum in an amount equivalent to 2.1 grams per cent, about three-fifths remained in the Howe albumin fraction, two-fifths precipitated out with the globulins; the latter being divided between the "pseudoglobulin I" and "II" fractions in the proportions 3 : 2. Of the Bence-Jones protein from the urine of Case 32 added to normal serum in an amount equivalent to 1.5 grams per cent, about 90 per cent was precipitated out with the globulins; about one-half of the latter appearing in the "euglobulin" fraction, one-fourth in the "pseudoglobulin I" fraction and one-sixth in the "pseudoglobulin II" fraction. We have not been able as yet to reproduce in this way large increases solely in the "pseudoglobulin

I" fraction. Nor have we been able to duplicate by such means large increases wholly or very largely in the "euglobulin" fraction, as anticipated from the studies of Magnus-Levy (6, 45) who showed that the "euglobulin" fraction in multiple myeloma usually contains little or no Bence-Jones protein.

The electrophoretic data on Bence-Jones proteins available to us included published values for three preparations (56) and unpublished values obtained (by E. A. K. while at Cornell Medical College) in a sample kindly furnished by Dr. V. DuVigneaud. Table IV includes additional data in 7 cases of multiple myeloma. Figure 5 shows the patterns obtained in Cases 11 (G) and 12 (H), the latter pattern indicating inhomogeneity.

It was found that, even under the uniform conditions of these determinations, the mobilities of the urinary Bence-Jones proteins of different patients differed widely—variations not wholly ascribable to differences in concentration, as indicated by dilution experiments. Using sodium phosphate buffer, pH 7.4,  $\mu = 0.1$ , the mobilities varied from 3.1 in Case 32 to 1.8 in Case 12 (Table IV).

Upon the addition of urine containing Bence-Jones protein to normal serum, the patterns obtained showed larger peaks at mobilities corresponding with the mobilities of the Bence-Jones proteins in the original urine samples. For example, addition of urine from Case 11 (containing Bence-Jones protein with mobility 2.8) resulted in an increase in the  $\beta$  component of normal serum (Figure 5J), also a corresponding further increase in the  $\beta$  component when the urine was added to a sample of the patient's serum. Addition of urine from Case 12 (containing Bence-Jones protein with mobility 1.8) resulted in an increase in the  $\gamma$  peak of normal serum (Figure 5K). Addition of urine from Case 3 (containing Bence-Jones protein with an intermediate mobility of 2.0) resulted in the appearance of an intermediate *M* peak in normal serum (Figure 5L). The  $\gamma$  and *M* peaks in Figure 5L are not completely separated because of the small difference in mobilities.

It was thus possible, by adding urinary Bence-Jones proteins to normal serum, and superimposing peaks due to Bence-Jones proteins upon

normal serum patterns, to reproduce in all essentials the several electrophoretic patterns encountered in the sera of our cases of multiple myeloma (*cf.* Figures 5D and J; A and K; E and L). The results further suggest that the apparent discrepancies between the results of Howe and electrophoretic analyses in some myelomatous sera are ascribable, in large part, to the presence of Bence-Jones proteins in those sera.

#### SUMMARY

Howe and electrophoretic analyses of the serum proteins were made in normal adults, in cases with hyperproteinemia due to various chronic infections or to cirrhosis, and in cases of multiple myeloma.

Results with the Howe method (carried out with minimal filtration error) are recorded in 36 normal subjects. The spread and the mean values, in grams per cent, were: For total proteins, 7.9 to 6.5 (7.2); for albumins, 5.7 to 4.7 (5.2); for total globulins, 2.5 to 1.3 (2.0); for "euglobulin," 0.4 to <0.1 (0.2); for "pseudoglobulin I," 1.9 to 0.8 (1.3); for "pseudoglobulin II," 0.8 to 0.2 (0.5). Electrophoretic patterns of normal human serum fractionally precipitated with sodium sulfate are presented.

Serum protein partitions by the Howe method are recorded in 10 cases of lymphogranuloma venereum, 10 cases of cirrhosis, 5 cases of sarcoid and 15 miscellaneous infections, all illustrating the distribution of Howe fractions in hyperglobulinemia. In these and in additional cases of similar etiology, hyperglobulinemia was found to be the result of a marked absolute and relative increase in the "euglobulin" fraction, associated with a less sustained absolute rise in "pseudoglobulin I"; the latter fraction showing a relative decrease in the higher globulin ranges. Marked hyperglobulinemia due solely to one or the other of these two fractions, or to "pseudoglobulin II," was not observed in this group. These relations, brought out particularly by graphic analysis, are compared with electrophoretic results in serum of high globulin content before and after fractional precipitation with sodium sulfate.

Serum protein studies by the Howe method in 38 cases of multiple myeloma are recorded. In some cases, hyperglobulinemia resulted from increase in both "euglobulin" and "pseudo-

globulin I" fractions, as in other diseases, though often in atypical proportions. In other cases anomalous results were obtained: the increase involved only the "pseudoglobulin I" fraction without any accompanying rise in "euglobulin," or the "pseudoglobulin II" or albumin fractions appeared to exceed maximal normal limits. Electrophoretic analyses in 10 cases gave similarly varied results: an increase in components moving with the mobility of  $\gamma$  globulins, of  $\beta$  globulins, or of globulins with intermediate mobilities forming a distinct  $M$  boundary. These anomalous Howe and electrophoretic patterns were found to be of value in diagnosis.

The significance of these peculiarities is considered in relation to the analogous salting-out and electrophoretic properties of urinary Bence-Jones proteins. It is shown that the broad zones of precipitation and the different mobilities of Bence-Jones proteins obtained from the *urine* of different cases of multiple myeloma correspond with the several anomalies observed in the Howe and electrophoretic fractionation of myelomatous *sera*. By adding urinary Bence-Jones proteins from different patients to normal serum, the essential characteristics of some anomalous Howe patterns and of all electrophoretic patterns observed in myelomatous sera could be reproduced.

Apparently, most myelomatous sera fall into one of three classifications: (1) Sera with hyperglobulinemia due to an increase chiefly in the Howe "euglobulin" fraction, partly in the "pseudoglobulin I" fraction; electrophoretically, to an increase in  $\gamma$  components. The globulin increment in these cases usually includes little or no Bence-Jones protein but is comparable to that observed in chronic infections. (2) Sera with or without hyperglobulinemia giving a variety of anomalous patterns by the Howe or electrophoretic methods, for the most part due to significant Bence-Jones proteinemia. It is not known whether in some such sera Bence-Jones proteins may occur in combination with other serum proteins. (3) Sera of apparently normal composition with respect to serum proteins.

We are indebted to the many physicians who permitted study of their cases of multiple myeloma; further to Dr. F. E. Kendall, Dr. L. G. Longsworth, Dr. T. Shedlovsky



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\* Indicates abstract of paper on program of American Society for Clinical Investigation.

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## ERRATUM

In the article entitled "Formulae for Afferent and Efferent Arteriolar Resistance in the Human Kidney: An Application to the Effects of Spinal Anesthesia" by Harold Lamport, which appeared in the September 1941 issue of this Journal on page 536, the following correction should be noted

### PART I

#### *Formulae*

$$H = \frac{I}{1 - H_c} \quad \text{should read} \quad H = \frac{1}{1 - H_c}$$

# MECHANISM OF DIURESIS: ALTERATIONS IN THE SPECIFIC GRAVITY OF THE BLOOD PLASMA WITH ONSET OF DIURESIS IN HEART FAILURE

By HAROLD J. STEWART

*(From the Department of Medicine of the New York Hospital and Cornell University Medical College and the Hospital of the Rockefeller Institute for Medical Research, New York)*

(Received for publication July 3, 1940)

There are divergent views concerning the mechanism by which diuresis is initiated. Many of the observations on this subject relate to mercurial drugs. Crawford and McIntosh (1) concluded that novasurol induced primary dilution, followed by concentration of the blood in edematous patients. Bryan, Evans, Fulton, and Stead (2) thought that salyrgan resulted in concentration of the blood, since sustained rise in its specific gravity occurred coincident with diuresis in dogs made edematous by plasmapheresis. Schmitz (3) did not find evidence that salyrgan mobilized fluid into the blood stream before the onset of diuresis. Blumgart, Gilligan, Levy, Brown, and Volk (4) concluded from observations on normal subjects that diuresis following xanthine and mercurial drugs was not initiated by the kidneys in response to measurable changes in specific gravity or sodium or chloride contents of the blood. Brown and Rowntree (5) found an increase in blood volume with the onset of diuresis in cardiac patients. On the other hand, using more accurate methods, Evans and Gibson (6) observed in dogs a diminution of plasma volume during diuresis induced by salyrgan.

For purposes of analysis the circulatory-renal system consists of the kidneys on the one hand and the tissues which retain fluid on the other, the circulating blood connecting them. Tissue fluid to be excreted as urine must be carried by the blood to the kidneys. The stimulus to inaugurate diuresis may be applied at different points in this system. If the mechanism is initiated in the kidneys and increased secretion of urine is the first step, concentration of the blood should occur on the withdrawal of fluid from it. How far this imbalance in the blood constituents can proceed and how long it can be present before restoration occurs by the entrance of fluid from the edematous tissues might vary with different diuretics.

Moreover, its duration may be brief before restoration is attempted, or it may be long enough and of such magnitude that it can be detected.

On the other hand, if diuresis is initiated at the tissue side of the system so that fluid enters the blood stream first, dilution of the blood would occur. Equilibrium would be disturbed until the kidneys began to excrete the surplus fluid. If dilution of the blood was of sufficient duration and magnitude, it might be detected.

It appeared that frequent observations of the specific gravity of the blood would reflect changes in dilution and concentration of the blood and give data which would contribute to one or the other of these points of view. Moore and Van Slyke (7) demonstrated a linear relationship between the observed specific gravity of the plasma of the blood and the observed protein content of the plasma of normal individuals and nephritic patients. They found also that when the specific gravity of the plasma was below 1.0223 in certain types of nephritis, edema was present, and that when the specific gravity was above this level, the so-called edema zone, edema did not occur.

In cardiac patients, Moore and Stewart (8) also found that the specific gravity of the plasma had a linear relationship to the protein content of the plasma and demonstrated that the plasma proteins and specific gravity of cardiac patients were above the edema level of nephritic patients. Moore and Stewart's (8) observations relating to the behavior of the specific gravity of the plasma of normal individuals provided the background for turning Moore and Van Slyke's method to this purpose. They found that the specific gravity of the blood plasma of normal individuals and of cardiac patients in water equilibrium remained constant to within 0.0004 from day to day. Dilution of blood by the injection of normal salt solution and following hemorrhage in dogs gave paral-

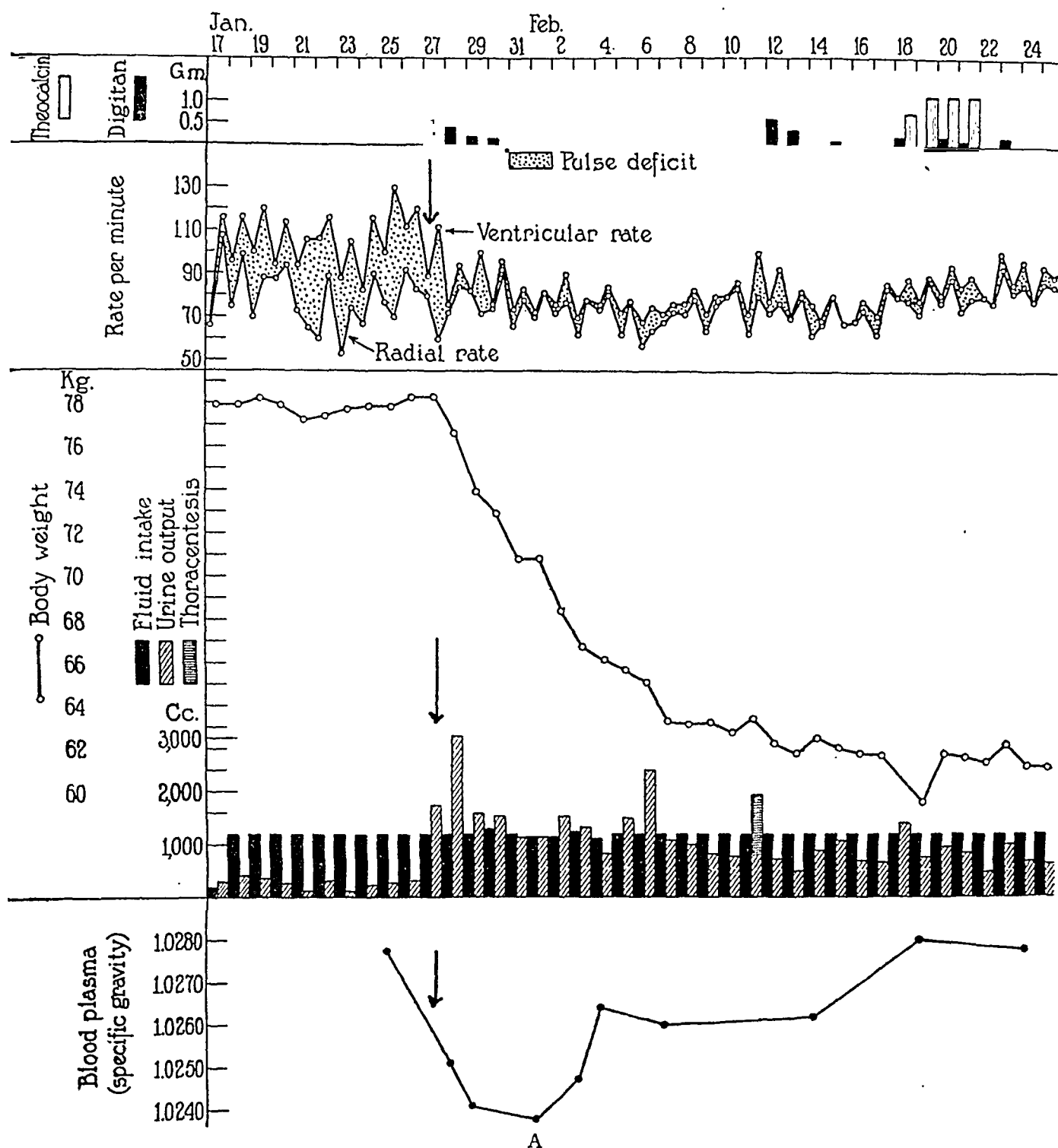


FIG. 1. RELATIONSHIP BETWEEN ONSET OF DIGITALIS, THEOCALCIN, AND SPONTANEOUS DIURESIS TO SPECIFIC GRAVITY OF THE BLOOD PLASMA

In this figure is shown the relation of onset of diuresis to the level of specific gravity of the blood plasma. In B. McL., 58 years (Figure 1A), in whom heart failure was a consequence of hypertension, diuresis resulted from giving digitan 1.2 grams in 24 hours. In L. C., 64 years (Figure 1B), in whom heart failure resulted from hypertension, the administration of theocalcin 4.5 grams on April 14, and daily thereafter, resulted in marked diuresis. In J. McM., 44 years (Figure 1C), in whom heart failure resulted from arteriosclerotic heart disease, diuresis occurred spontaneously.

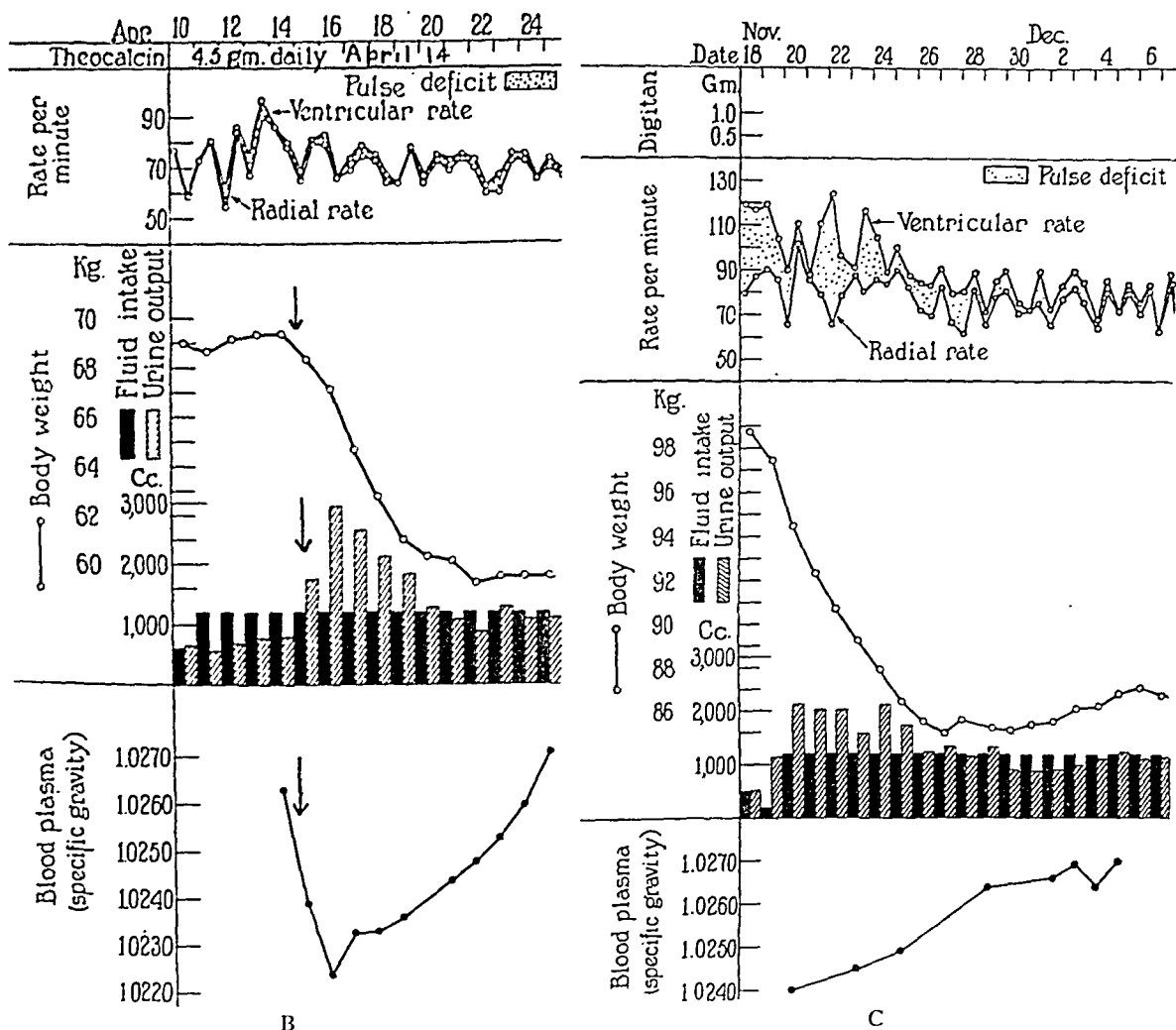


FIG. 1 (continued)

lel decreases in specific gravity of the plasma and plasma proteins. The oral administration of 1000 cc. of water did not alter specific gravity of the plasma; during the urine concentration test, however, the specific gravity and the plasma proteins increased, indicating concentration of the blood. In short, when concentration of the blood occurs, the specific gravity rises and when dilution occurs, it decreases.

It appeared from these observations that during a time in which nothing was done to deplete the plasma proteins, the specific gravity of the plasma could be used to indicate dilution or concentration of the blood.

#### METHODS AND PLAN OF OBSERVATIONS

The specific gravity of the plasma of the blood of cardiac patients exhibiting signs and symptoms of con-

gestive heart failure was estimated by the method of Moore and Van Slyke (7). Duplicate samples were checked to within 0.00002. The daily fluid and salt intakes of the patients were 1200 cc. and 5 grams, respectively. Patients remained in bed. There was a control period before giving diuretics. Since samples of blood were taken frequently before and after the administration of diuretics, it was not expedient to keep the subjects in the basal or postabsorptive state. The patients ate breakfast at 7:30 a.m., after which they received no fluid<sup>1</sup> until samples of blood were taken without stasis at 10:30 a.m. The diuretic was then given. A rubber stopper was used to eliminate evaporation. Clotting was prevented by use of heparin.

<sup>1</sup> It is recalled, however, that Moore and Stewart (8) found that drinking 1000 cc. water did not alter the specific gravity of the blood.

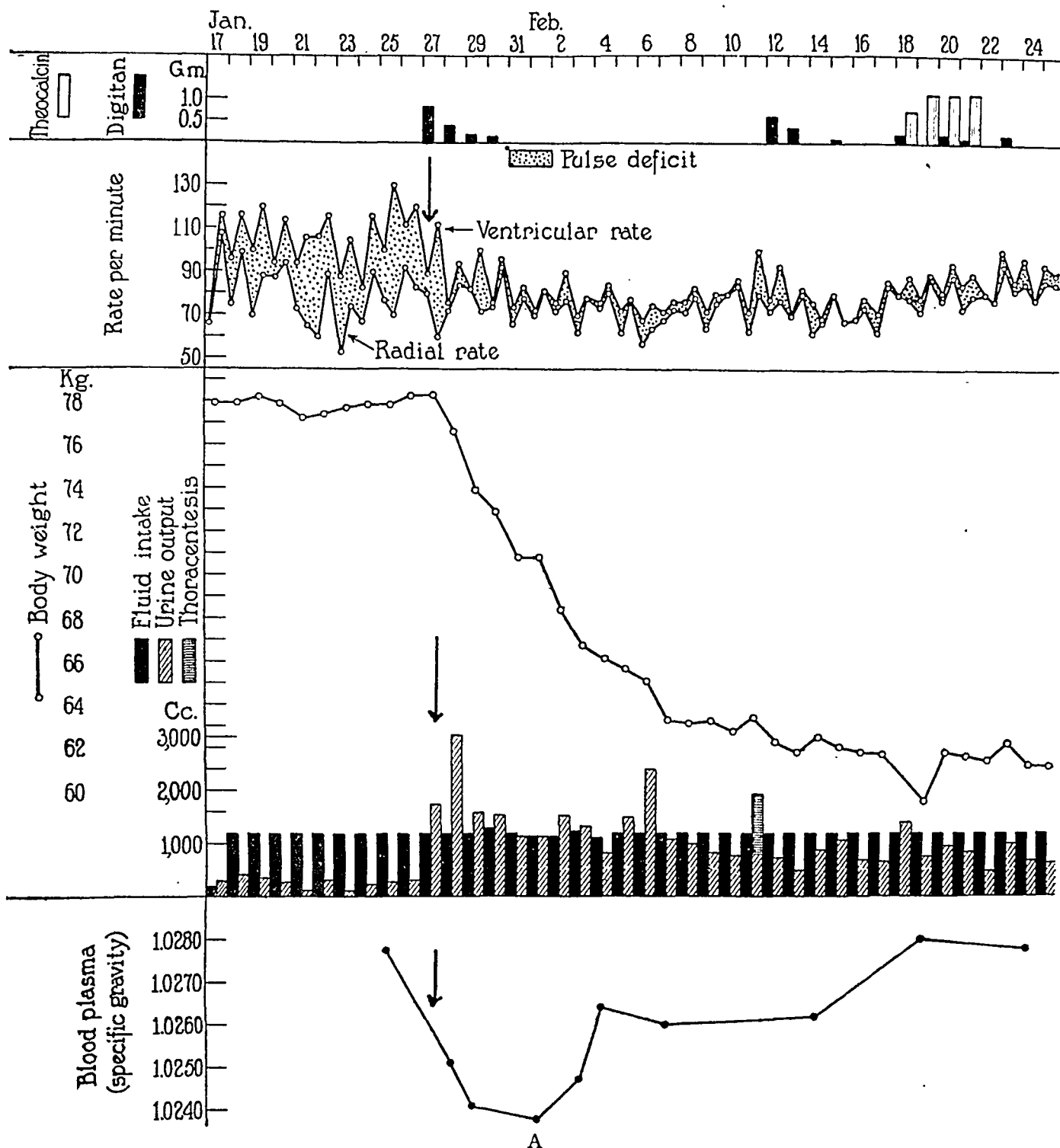


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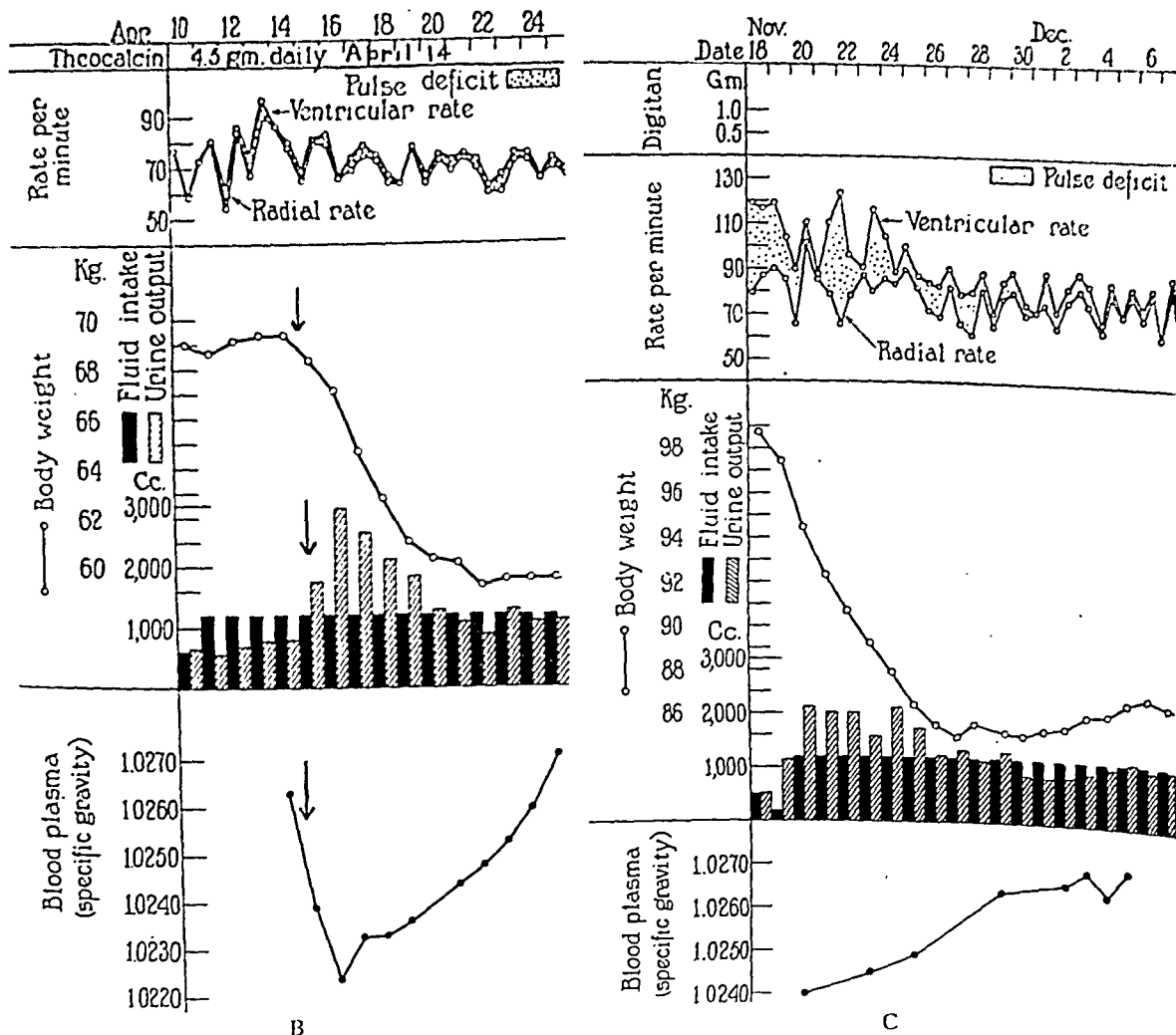


FIG. 1 (continued)

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#### METHODS AND PLAN OF OBSERVATIONS

The specific gravity of the plasma of the blood of cardiac patients exhibiting signs and symptoms of con-

gestive heart failure was estimated by the method of Moore and Van Slyke (7). Duplicate samples were checked to within 0.00002. The daily fluid and salt intakes of the patients were 1200 cc. and 5 grams, respectively. Patients remained in bed. There was a control period before giving diuretics. Since samples of blood were taken frequently before and after the administration of diuretics, it was not expedient to keep the subjects in the basal or postabsorptive state. The patients ate breakfast at 7:30 a.m., after which they received no fluid until samples of blood were taken without stasis at 10:30 a.m. The diuretic was then given. A rubber stopper was used to eliminate evaporation. Clotting was prevented by use of heparin.

<sup>1</sup> It is recalled, however, that Moore and Stewart (8) found that drinking 1000 cc. water did not alter specific gravity of the blood.

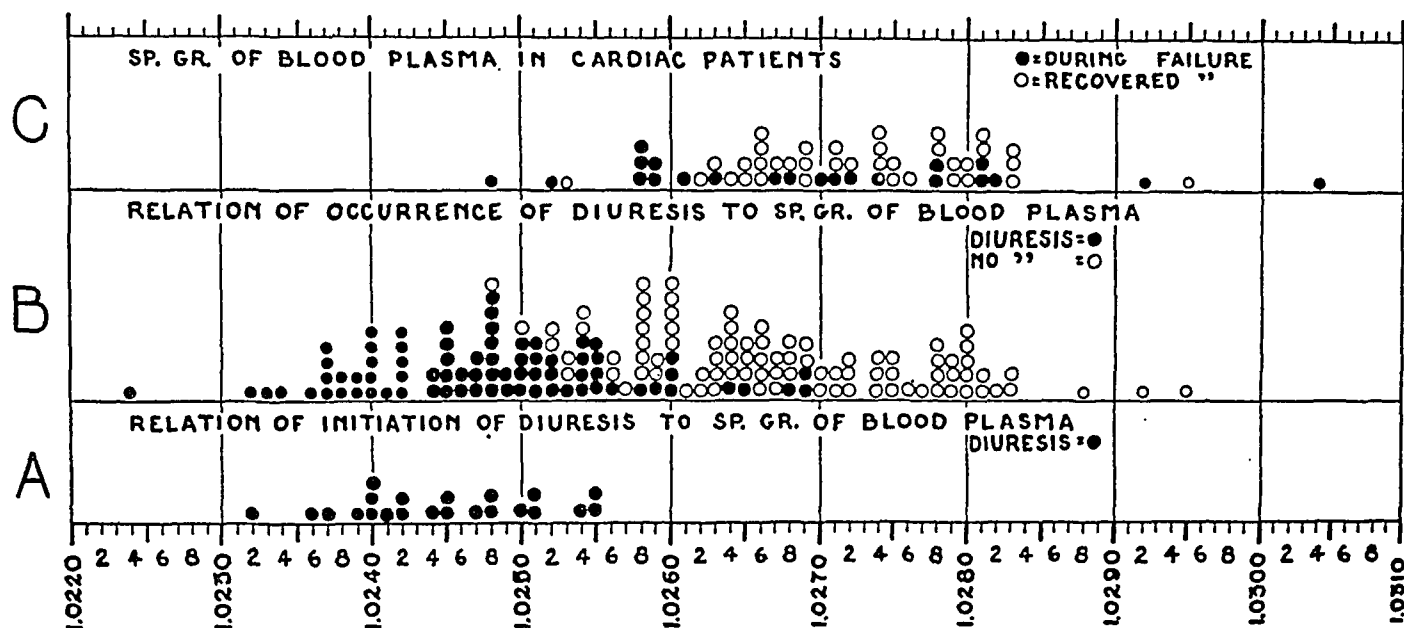


FIG. 2. RELATION OF INITIATION AND OCCURRENCE OF DIURESIS TO SPECIFIC GRAVITY OF THE BLOOD PLASMA

In Figure 2A are plotted specific gravity estimations of the plasma to show the level at which diuresis was initiated. Closed circles indicate that diuresis was initiated at the level shown. In Figure 2B are plotted data to show the relation of occurrence and persistence of diuresis to specific gravity of the blood plasma. Closed circles indicate occurrence of diuresis and open ones that diuresis was not taking place. In Figure 2C are plotted specific gravity estimations of patients during (closed circles) and recovered (open circles) from heart failure.

#### OBSERVATIONS

Specific gravity of the blood plasma was observed in instances of diuresis occurring spontaneously without the use of drugs (8 patients), in diuresis resulting from digitalis (9 patients), and, finally, in diuresis following the use of theocalcin (Merck) (5 patients). Patients having auricular fibrillation, as well as those with normal sinus rhythm, were subjects.

Patient B. McL. (Figure 1A) illustrates the effect of digitalis. Rest in bed and restriction of fluids did not result in diuresis. When digitan 1.2 grams (Merck)<sup>2</sup> were given in 24 hours, diuresis with loss in weight occurred promptly. The ventricular rate decreased. On the first day of diuresis, the specific gravity of the blood plasma fell to 1.0250 from a control level of 1.0280, and the next day, with further increase in urine output, to 1.0230, and then rose gradually to 1.0280 again. Similar results were seen in 7 patients with auricular fibrillation and in 2 patients with normal sinus rhythm. In an eighth patient with auricular fibrillation, in whom diuresis did not occur, specific gravity of the plasma did not fall.

The case of L. C. (Figure 1B) illustrates the effect of theocalcin. The specific gravity of the plasma was 1.0264 during the control period; with 4.5 grams of theocalcin daily, diuresis occurred promptly; the specific gravity fell immediately to 1.0239 and then further the next day to 1.0223 and rose gradually to 1.0275 toward the end of

diuresis. Similar patterns were recorded in one other patient with auricular fibrillation and 3 patients with normal sinus mechanism.

The case of J. McM. (Figure 1C) illustrates the results in 3 patients with auricular fibrillation and 5 patients with normal sinus rhythm in whom diuresis occurred spontaneously with rest in bed and limitation of fluid intake. There was a fall in specific gravity when diuresis occurred; or, if diuresis had been initiated when the first sample was taken, the specific gravity was low, rose with the continuance of diuresis, and had risen to a normal level when excess fluid had been eliminated.

For all patients the level of specific gravity of the plasma was plotted to show the onset of diuresis (Figure 2A). When it fell to 1.0250 in 17 instances and to 1.0255 in 5 instances, diuresis was initiated; above 1.0255 the onset of diuresis was not observed.

For all patients the level of specific gravity of the blood plasma was plotted to show not only the onset but also the persistence of diuresis (Figure 2B) after it had been initiated. Below 1.0248, diuresis either occurred or was initiated in every instance, and in the zone 1.0248 to 1.0255, there were only a few instances in which diuresis was not occurring. Diuresis, having been initiated at a specific gravity below 1.0255, may continue with a rising specific gravity, even when it has attained values up to 1.0269. The value 1.0255 may be called the critical level for the initiation of diuresis in congestive heart failure.

The specific gravity of the plasma of 13 cardiac patients, in water equilibrium during congestive heart failure, ranged from 1.0248 to 1.0304 (Figure 2C). In one subject only it was at this lower level, and in 3 instances

<sup>2</sup> This amount of this preparation decreased the ventricular rate in auricular fibrillation to around 70 per minute when given in 24 hours and was called the digitalizing amount.

only was it below 1.0258. In short, all values were in the normal range and above the edema level of 1.0233 found for patients with nephritis (7). Moreover, these values are above the diuresis level which was observed in patients in this study. The specific gravity of the plasma of 16 cardiac patients, in water equilibrium and recovered from congestive heart failure, ranged from 1.0253 to 1.0295 (Figure 2C), values similar to those recorded during failure.

#### DISCUSSION

Data now available show that the specific gravity of the blood plasma of cardiac patients, whether before the occurrence of failure (8), during failure, or after recovery from failure, when the subjects are in water equilibrium, is in the normal range, 1.0248 to 1.0304; the occurrence of edema in them does not depend on the level of specific gravity of the plasma or of the plasma proteins. When theocalcin or digitalis was given and diuresis occurred, the specific gravity of the plasma fell from a higher level to 1.0255 or lower (Figures 1A, 1B, 2A) and, in all except 6 instances, fell below 1.0248. Once diuresis had been initiated, it could proceed with a specific gravity above these levels as the specific gravity returned toward or above the original levels (Figure 2B). The pattern was similar when diuresis occurred spontaneously. These observations indicate that fluid is drawn into the blood, giving rise to dilution of the blood and by inference to increase in its volume. Since this was the sequence on each occasion when diuresis occurred, the inference may be drawn that increase in blood volume was the stimulus which led the kidneys to begin diuresis. When the specific gravities of frequent samples of blood taken before and after the giving of these drugs were correlated with the voiding of urine, it was observed that a fall in specific gravity of the plasma preceded the occurrence of diuresis. The occurrence of diuresis could be predicted when it was discovered whether the specific gravity fell or was unchanged. In no instance did the specific gravity rise with onset of diuresis.

In patient E. G. the specific gravity of the plasma before the onset of diuresis was 1.0270. The specific gravity of the edema fluid, removed from the legs by Southey tubes and collected under oil to avoid evaporation, was 1.0080. With the initiation of diuresis 3 days later, the specific grav-

ity of the plasma fell to 1.0240. By the addition of varying amounts of edema fluid to the blood plasma and estimation of the specific gravity of the mixture, it was calculated that the addition of approximately 400 cc. of edema fluid having a specific gravity of 1.0080 to the blood would have been sufficient to reduce the specific gravity to the observed level of 1.0240. The blood volume was presumably increased by this amount. Estimation of the urinary excretion of protein in a few patients during diuresis did not account for the low concentration indicated by the fall in specific gravity, and eliminated the possibility that there had been loss of proteins from the blood by this channel. The decrease was thought to be due not to loss of protein from the blood, but to dilution of the blood by edema fluid or by other fluid accumulations. It is to be emphasized that diuresis did not occur because the plasma proteins had been lowered. The fall in specific gravity from a high level to a low one represented increase in blood volume with dilution of the blood; in short, sufficient increase in blood volume had occurred by change from the high specific gravity to 1.0255, or lower, to provide the stimulus for the kidneys to initiate diuresis.

These results, in diuresis occurring spontaneously as well as following digitalis and theocalcin, are similar to those Crawford and McIntosh (1) found with novasurol. They differ, however, from Evans and Gibson's (6) findings in dogs. The divergent results may be due in part to the difference of the subjects in the two studies (patients suffering from heart failure in one, and dogs rendered edematous by plasmapheresis in the other) and in part to different drugs.

The normal level of specific gravity of the plasma in cardiac patients not only during failure but after recovery from failure demonstrates that the occurrence of edema is not dependent upon lowered plasma proteins. These results are in agreement with those of Moore and Stewart (8). On the other hand, Thomson (9), Ellis (10) and Payne and Peters (11) have found low plasma proteins in patients with cardiac edema. The data now being recorded show that the specific gravity of the plasma and the plasma proteins decrease with onset of diuresis. It may be that certain of Thomson's (9) and Ellis' (10) and Payne and Peters' (11) patients were not in water



equilibrium when observations were made; diuresis may have been about to start or was already occurring. Data relating to urine output and changes in body weight were not available in their papers for making an analysis from this point of view. No doubt, in certain cardiac patients plasma protein deficiency from low protein intake or as the result of cardiac cirrhosis may also be operative as an additional factor, but it does not appear to play a rôle in the causation of edema in the uncomplicated case of heart disease with failure.

#### SUMMARY

(1) Change in level of the specific gravity of the plasma has been used as a measure of change in blood volume, decrease in specific gravity indicating dilution of the blood, and increase in specific gravity concentration. It appeared that diuresis in the presence of heart failure of the congestive type depended on changes initiated in the tissues, since it was accompanied by decrease in specific gravity of the plasma, that is to say, by dilution of the circulating blood with increase in blood volume. Dilution of the blood preceded the onset of diuresis and increase in blood volume appeared to be the stimulus that initiated diuretic response of the kidneys. The results were similar not only when diuresis occurred spontaneously, but when it was occasioned by digitalis and by theocalcin and, moreover, whether in the presence of normal sinus rhythm or of auricular fibrillation, and whether the cause of the heart disease was rheumatic fever, syphilis, arteriosclerosis or hypertension. These studies do not relate to the anatomical portions of the kidney which take part in the accelerated formation of urine in diuresis.

(2) The specific gravity of the plasma must fall from a high level to 1.0255 or lower for the initiation of diuresis. Below this level may be called the diuretic zone in heart failure, in that it corresponds to a dilution of the blood with increase in blood volume of sufficient magnitude to initiate diuresis.

(3) In uncomplicated heart disease the level of specific gravity of the plasma, and by inference the plasma proteins (7, 8), is in the normal range not only before the onset of failure (8) but dur-

ing and after recovery from failure. Plasma protein deficiency does not participate in the etiology of cardiac edema usually.

(4) In the technique of taking blood for plasma or serum proteins or specific gravity of the plasma, samples should not be obtained when diuresis is being initiated or occurring, since low results may be recorded which do not reflect the usual level for the subject. Results should be interpreted in the light of the effect of diuresis which has been demonstrated in this investigation.

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# THE URINARY/FECAL COPROPORPHYRIN RATIO IN LIVER DISEASE

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(Received for publication July 11, 1940)

In addition to the bile pigments, there are present in the urine and stool another series of pyrrole pigments, the porphyrins. Hemoglobin, myoglobin, and other respiratory pigments contain as their prosthetic group a porphyrin of Type III configuration. The porphyrin excreted in urine and feces by normal individuals, and in most pathological states, is coproporphyrin Type I. This substance cannot be derived by degradation of the Type III porphyrins present in the respiratory pigments (1). Dobriner and his associates have shown that coproporphyrin I is formed as a by-product in the course of hematopoiesis (2a, b, c). Furthermore, they showed that the rate of production and excretion of coproporphyrin I depends upon the activity of orderly hematopoiesis (3a, b, c). Dobriner (4a, b) and Watson (5a, b, c, d, e) have established that the excreted coproporphyrin is chiefly, if not entirely, endogenous in origin. The greater proportion of the coproporphyrin is excreted by the liver into the intestinal tract and is found in the stool. There is a similarity between the pathways of excretion of coproporphyrin and the bile pigments.

Salkowski (7) and Garrod (9), in their early work on porphyrins, observed an increased urinary output of porphyrins in liver disease. Elevated urinary porphyrin excretion has been recorded in cases of passive congestion of the liver, cholangitis, catarrhal jaundice, luetic hepatitis, hemachromatosis, secondary carcinoma of the liver, acute and subacute liver atrophy, and cirrhosis (4a, 6, 10a, b, 11a, b, 12, 13, 15, 16, 17, 18).

No suitable fecal coproporphyrin studies have been reported in cases of diseases of the liver. Brugsch (11a, b) and Vigliani (6) did not separate the fecal porphyrins into their components, hence their quantitative data for fecal porphyrin excretion comprise the total fecal porphyrins, and include deuteroporphyrin and protoporphyrin, which are partially exogenous in origin (4b, 5e).

Brugsch (11a, b) suggested that there might be value in the determination of the relative excretion of urinary and fecal porphyrins in cases of liver disease.

It was reasoned that, since the coproporphyrin is excreted by the liver as well as by the kidney, the injured liver might be unable to excrete the total amount of coproporphyrin presented to it, and therefore this substance would accumulate in the blood stream and be excreted in the urine. Thus the urinary excretion would be increased at the expense of the fecal output. In cases of liver insufficiency, the ratio of urinary to fecal coproporphyrin should be elevated. In order to determine the amount of porphyrin cleared by the liver, only the coproporphyrin need be determined, since it alone is excreted in both urine and feces.

## METHODS

The quantitative separation methods for coproporphyrin used in our studies are those of Dobriner (4a, b, 20). Quantitative measurements were done by means of a spectroscopic colorimeter (21).

All of the patients studied were on a regular diet. The entire urine and stool were collected for a period of seventy-two hours. In order to insure accurate collections of feces, capsules of brilliant blue were administered at the beginning and end of the seventy-two-hour test period, and the stool beginning at the first marker and ending just in front of the second marker was taken as a seventy-two-hour specimen. Actual determinations were done on aliquots of the stool and urine. The coproporphyrin output of the urine and stool was determined and the ratio of urinary to fecal coproporphyrin computed for a twenty-four-hour period. All cases studied were tested for the presence of bile pigments in the stool, and found to be positive.

## RESULTS

Studies were done on a total of twenty-five cases. Of these, five (Cases 1 to 5, Table I) are in the normal group and the ratio of urinary to fecal coproporphyrin varied from 0.3 to 0.6. One

equilibrium when observations were made; diuresis may have been about to start or was already occurring. Data relating to urine output and changes in body weight were not available in their papers for making an analysis from this point of view. No doubt, in certain cardiac patients plasma protein deficiency from low protein intake or as the result of cardiac cirrhosis may also be operative as an additional factor, but it does not appear to play a rôle in the causation of edema in the uncomplicated case of heart disease with failure.

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## METHODS

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## RESULTS

Studies were done on a total of twenty-five cases. Of these, five (Cases 1 to 5, Table I) are in the normal group and the ratio of urinary to fecal coproporphyrin varied from 0.3 to 0.6. One

of the normals (Case 5), a 27-year-old male in good general condition, was suffering from an inguinal furuncle with adenitis and had moderate fever during the course of the test. The total excretion in this case was elevated (585 gamma), although the ratio was within normal limits.

TABLE I  
*Coproporphyrin excretion in normals*

Case	Age	Sex	Urine copropor- phyrin	Fecal copropor- phyrin	Total copropor- phyrin	Ratio
1	25	F	50	150	200	0.3
2	26	M	130	205	335	0.6
3	28	M	95	285	380	0.3
4	29	M	150	245	395	0.6
*5	27	M	220	365	585	0.6

\* Fever.

Ten of the patients (Cases 10 to 19, Table III) fall in the category of cirrhosis. Two of these were of the juvenile portal type, one of the cardiac type, and the remainder of the classical portal variety. The diagnosis in five of these cases was verified by autopsy, biopsy, or peritoneoscopy. The urinary/fecal coproporphyrin ratio in these cases varied from 0.8 to 12.0.

One patient with a ratio of 0.3 (Case 20, Table III) was convalescing from catarrhal jaundice, and manifested normal bromsulfalein and galactose tolerance tests.

Of the remaining nine cases, three (Cases 21 to 23, Table III) had secondary carcinoma of the liver, one of these being verified by biopsy. The

urinary/fecal coproporphyrin ratios in these cases were 0.8, 20.0, and 22.0.

A patient with a ratio of 4.3 (Case 24, Table III) was classified as essential xanthomatosis (22); and another (Case 25, Table III), with a ratio of 13.3, was diagnosed as subacute liver atrophy.

Three patients (Cases 6, 7, 8, Table II) had doubtful liver insufficiency, although all had enlarged livers. The ratios in these patients varied from 0.45 to 0.7. The final case (Case 9, Table II), clinically designated as cirrhosis of the liver, had a normal bromsulfalein excretion and blood chemistry. This patient was operated upon. Liver tissue was not obtained for examination, although a portion of the greatly thickened capsule was examined. The diagnosis of the pathologist was "Chronic inflammation of the capsule of the liver which in some respects corresponds to the thickening seen in polyserositis." On reviewing this case, who had an ascites of three years' duration, it was found that she had had a previous operation for adhesive pericarditis. In spite of the clinical diagnosis of cirrhosis, we feel that this is not a case of parenchymal liver disease but one of polyserositis.

#### DISCUSSION

The coproporphyrin output in several normal males was determined by Dobriner, Strain, and Localio (2a). The total output varied between 306 and 376 micrograms per diem, of which 64 to 123 micrograms were excreted in the urine.

TABLE II  
*Coproporphyrin excretion in hepatomegaly without clinically disturbed liver function*

Case	Age	Sex	Diagnosis	Red blood cells	Hemo- globin	Ic- terus index	Serum bili- rubin	Cho- les- terol	Cho- les- terol esters	Ratio choles- terol esters/ choles- terol	Brom- sulf- alein	Urine copro- porphyrin	Fecal copro- porphyrin	Total copro- porphyrin	Ratio urinary/ fecal copro- porphyrin
6	55	F	Pyelonephritis hepatomegaly	millions 3.94	grams 12.3	5				per cent	per cent 16 12	190	370	560	0.5
7	67	M	Myxedema hepatomegaly	3.27	9.8	4	very faint trace	235	95	41	18 trace	50	70	120	0.7
8	18	F	Rheumatic heart dis- ease, hepatomegaly	4.02	13.5			170	70	41	8 trace	45	115	160	0.45
9	40	F	Polyserositis, ascites	3.84	12.0			190	60	32	8 6	35	100	135	0.35

TABLE III

*Coproporphyrin excretion in cases with disturbed liver function*

Case	Age	Sex	Diagnosis	Red blood cells millions	Hemoglobin grams	Icterus index	Serum bilirubin	Cholesterol	Cholesterol esters	Ratio cholesterol esters/cholesterol per cent	Brom-sulfalein 5 mgm. dose 30 and 60 minutes per cent	Galactose tolerance, Takata Ara	Urine coproporphyrin	Fecal coproporphyrin	Total coproporphyrin	Ratio urinary/fecal coproporphyrin
10	19	M	Portal cirrhosis, juvenile (biopsy)	4.60	14.0	9.4	less than 1.0	195	80	41	18 12	negative/negative	145	135	280	1.1
11	18	F	Portal cirrhosis, juvenile (peritoneoscopy)	4.00	13.9	22.0	2.0	210	60	28	40 28	7.04 grams	310	250	560	1.2
12	46	F	Cardiac cirrhosis	4.42	12.6	6.8		170	trace	low			100	115	215	0.9
13	60	M	Suppurative cirrhosis (biopsy)	4.56	13.5	10.0	less than 1.0	230	65	28	20 12	negative	75	95	170	0.8
14	34	M	Portal cirrhosis			35.7	4.5	200			40 40		150	165	315	0.9
15	55	M	Portal cirrhosis	4.36	9.9	10.7		130	trace	low	38 28		110	85	195	1.3
16	55	M	Portal cirrhosis	4.39	14.0	13.6	less than 1.0	375	180	48	40 28	3.2 grams/negative	90	45	135	2.0
17	48	F	Portal cirrhosis	2.84	10.2	33.8		250	45	18	40 24	negative	125	55	180	2.3
18	33	M	Portal cirrhosis (autopsy)	4.62	14.0	125.0	26.0	180	trace	low	4+ 4+	4.2 grams/positive	960	80	1040	12.0
19	63	M	Portal cirrhosis (autopsy)			34.1	3.8	335	70	21	36 32		400	70	470	5.9
20	21	F	Convalescent catarrhal jaundice	4.92	14.6	12.5					6 6	negative	50	185	235	0.3
21	47	F	Secondary carcinoma of liver (biopsy)	2.76	9.3	10.0	less than 1.0	230	65	28	20 12	negative	75	95	170	0.8
22	29	M	Secondary carcinoma of liver	3.46	11.9	40.9	7.3	355	115	32	40 28		500	25	525	20.0
23	51	M	Secondary carcinoma of liver	3.54	12.8	136.0	15.0	305	40	13	4+ 4+		220	10	230	22.0
24	44	F	Essential xanthomatosis	3.20	11.0	83.0	12.0	360	125	35	40 36		280	65	345	4.3
25	22	M	Subacute liver atrophy	4.40	15.5	158.0	24.7	180	32	16	40 40		200	15	215	13.3

We have calculated the urinary/fecal coproporphyrin ratios in these cases and found them to vary from 0.2 to 0.6. In our own group of cases, the urinary/fecal coproporphyrin ratio in normals varied from 0.3 to 0.6.

In normal females the total excretion is slightly less but the ratio is unchanged. The lowered total excretion manifested by females is illustrated by Cases 1, 8, 12, and 20.

Dobriner and Rhoads (8), in their review of the porphyrins have stated that the total coproporphyrin I output is dependent on several factors, the principal one being the activity of the hematopoietic system.

In Tables II and III there are several cases exhibiting abnormally low total excretion. Six of these cases (7, 9, 15, 17, 21, 23) are seen to present secondary anemia. Two others (13, 16)

have low excretory values which cannot be adequately explained.

Increased total coproporphyrin output in febrile states is illustrated by Cases 5 and 6. In these cases, although the total excretion was increased, the ratios were within the range of normal. It is possible that the increased excretion in the presence of fever is due to the stimulating effects of elevated temperatures upon hematopoietic activity. With the exception of these two, all cases studied were afebrile during the course of the tests.

Dobriner (4a, b) and Vigliani and Libowitzky (23) have demonstrated the excretion of coproporphyrin III in a number of cases of liver disease such as melanocarcinoma, acute and subacute liver atrophy, and in some cases of cirrhosis. No attempt was made in the present study to ascertain whether coproporphyrin III was excreted in any

of the cases, because sufficient material for melting point determination was not available. However, the high values obtained in four of the cases (Cases 11, 18, 22 and 24), in which there was no evidence of hyperactivity of the bone marrow and in which fever was not present, are possibly attributable to the excretion of coproporphyrin III in addition to the usual coproporphyrin I.

Because of these many divergent factors, the importance of determination of the urinary/fecal coproporphyrin ratios is emphasized in evaluating the status of the liver. It can readily be seen that a patient might exhibit a high urinary coproporphyrin excretion which would lead one to false conclusions in the absence of fecal coproporphyrin studies. However, the fecal coproporphyrin excretion might be at such a level that a normal ratio obtains, *e.g.*, Case 5 with a urinary excretion of 220, and Case 6 with a urinary coproporphyrin excretion of 190. The converse may also be true, in that a case with a normal urinary coproporphyrin excretion with a low total output and low fecal coproporphyrin excretion may be included as normal, although the calculated ratio may be elevated, *e.g.*, Cases 12, 13, 15, 16, 17, 21.

In the tests of liver function done concomitantly with the porphyrin excretion studies, it appears that the ratio of urinary/fecal coproporphyrin output more closely approximates the clinical evaluation of disturbance of liver function and the blood chemistry changes than do certain of these tests. The cholesterol ester/total cholesterol ratio was normal in five of the undoubted cases of liver insufficiency (Cases 10, 16, 21, 22, 24). In six of the fifteen abnormal cases studied, galactose tolerance tests were performed, and in three of these, all cases of liver insufficiency, the tests were within the range of normal. The Takata Ara test, done in three cases, was negative in two. Abnormal bromsulfalein retention was observed in all of the fifteen cases, although the retention in some of the cases does not appear to parallel the degree of liver insufficiency.

Since the procedure is dependent upon the integrity of the extra-hepatic biliary passages, it is obvious that it cannot be used in cases with obstruction of these passages.

## CONCLUSIONS

(1) In the normal individuals studied, the ratio of urinary to fecal coproporphyrin varied from 0.3 to 0.6.

(2) In cases of liver insufficiency this ratio was increased.

(3) The highest ratios were obtained in the cases exhibiting the most evident disturbance of liver function.

The authors wish to express their gratitude to Dr. Konrad Dobriner for the interest shown during the progress of this work.

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# STUDIES ON THE RELIEF OF PAIN BY COUNTERIRRITATION

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Counterirritation has always been one of the most important methods available to physicians for the relief of pain, yet it is poorly understood and in recent years has claimed scant attention. Our purpose was to experience, study, and analyze the phenomena of counterirritation in the hope of obtaining information which would lead to the improvement of the clinical methods now in use.

The extreme difficulty of evaluating a patient's statements regarding the discomfort from which he is suffering need not be emphasized. Therefore, it seemed wise to begin by inducing pain in ourselves and to experience the effects of the various forms of counterirritation upon it. The larger part of this communication is a report of such experiences. Preliminary reports have already appeared (1).

In this way the stimuli commonly used in counterirritation—heat, cold, rubbing, scratching, etc.—were tested and their relative effectiveness asayed. Also, the utility of electrical stimulation of varying strength, duration, and frequency, was investigated.

Later we performed experiments designed to give insight into the physiological mechanism of the relief secured; first on ourselves, and second, with the help of Dr. D. W. Bronk, on animal preparations.

Finally, we attempted to make some use of the information gained by applying it to relieve the pains of over 80 patients. This phase of our work merits a preliminary report only. But the results have convinced us that the ordinary clinical methods of relieving pain by counterirritation are far from the best which can be devised.

## TECHNIQUE

### *Methods employed to induce pain in ourselves*

(1) *Application of irritants to the skin.* When an ointment containing 3 per cent capsicum,<sup>1</sup> 10 per cent turpentine and certain other irritants was rubbed into the skin, burning pain began in about 10 minutes and lasted several

<sup>1</sup> Capsolin from Parke Davis and Co.

hours. By large dosage and vigorous rubbing the discomfort was made as great as could be tolerated. The pain could, however, be ended by repeated applications of petrolatum rubbed into the area and then wiped off.

(2) *Subcutaneous injection of irritating solutions.* A 10 per cent sterile solution of NaCl proved most satisfactory for our purpose. Injection of amounts up to 0.25 cc. could be tolerated. The violent pain which followed immediately was characteristically a deep seated ache with an occasional more severe "shooting pain" and was sometimes accompanied by tingling. This pain lasted with little diminution of intensity for about 20 minutes and then slowly subsided; experiments were possible for about 30 minutes. Tenderness and induration persisted for about 24 hours. In one of us (S) the pain remained localized, in the other (G) it radiated markedly up and down the arm and to the opposite surface.

### *Methods of counterirritation*

(1) *Temperature changes* were secured by application of an ordinary rubber hot-water bottle or ice bag. In some experiments measurements of skin temperature were made by means of a thermocouple.

(2) *Vibration* was secured by an alternating current vibrator which produced a movement of about 1 mm. 60 times a second.

(3) *Tactile sensations* were induced by rubbing the skin with cotton for mild effects and by scratching it with a sharp wooden edge for stronger effects. An air jet was used in a few experiments to secure rapidly interrupted stimulation. Both a fan and a source of compressed air were used.

(4) *Electric stimulation* was usually secured by a stimulator, designed and constructed by Mr. J. P. Hervey, which permitted alteration in the strength, frequency, and duration of the stimuli. A Harvard inductorium was also occasionally employed. The stimulating electrode consisted of a pad of cotton moistened with normal salt solution and the indifferent electrode was a large copper plate.

(5) *Static electricity* generated by a Wimhurst machine of the type commonly used in the treatment of hysterical manifestations was employed in some experiments.

### *Conduct of experiments on ourselves*

The subject sat with his arm extended on a table and took careful note of the variations in intensity and character of the pain produced in the flexor surface of the forearm by one of the methods mentioned above. A few minutes were allowed for the discomfort to become

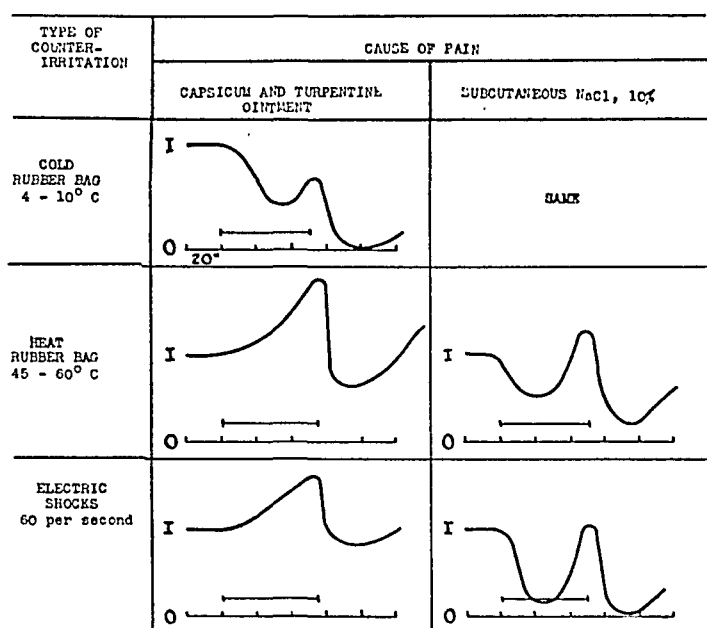


FIG. 1. EFFECT OF COUNTERIRRITATION ON PAIN INDUCED BY SKIN IRRITANTS AND BY SUBCUTANEOUS 10 PER CENT NaCl (LEFT AND RIGHT COLUMNS, RESPECTIVELY).

Ordinate is the amount of pain, I representing the initial intensity, and O no pain. Abscissa time in 20-second intervals. The duration of counterirritation is indicated by the horizontal bar. The top row shows the result of the application of cold; the middle, of hot water in rubber bags. The lower row gives results obtained after electric shocks at a rate of 60 per second.

constant before counterirritation was applied. The original pain was arbitrarily graded at 100, and subsequent pain was assessed in percentage of the original.

Our experience consisted of some 50 applications of painful stimuli and over 200 tests of various types of counterirritation. On three occasions the effect of counterirritation on spontaneous headache in one of the authors was studied.

## RESULTS

### *Deductions drawn from experiments on the authors*

*General pattern of the response.* Irrespective of the type of painful stimulus, or of the counterirritant, the effect of the latter on the sensation of pain had a characteristic pattern. On the application of a counterirritant the pain was decreased or abolished immediately, but it soon began to return slowly, and within a minute had regained its original intensity even though counterirritation had been continued. If the counterirritant was then removed, prompt relief of the pain followed again; this relief was also temporary and within a minute the pain came back to its original in-

tensity. Figure 1 gives typical examples of this pattern.

The pattern described above did not occur invariably. Mild pains were often permanently relieved and did not recur during the counterirritation; the severest pains were not relieved at all. The typical pattern was generally found in pains of moderate severity; in these temporary relief following the application of the counterirritant was the rule, but the relief following its removal occurred in only about three-fourths of the experiments.

One other subjective phenomenon occurred so frequently that it deserves mention. Immediately following the application of counterirritation a sharp, but very transient, increase in pain frequently preceded the relief. A similar transient increase was often noted before the relief which followed removal of counterirritation.

*Relation between intensity of counterirritation and the relief produced.* This relation was tested with cold, heat, tactile, and electrical counterirritation, and a general statement concerning the results seems warranted. The most effective intensity of counterirritation was slightly less than that which produced manifest discomfort when applied to normal skin. If counterirritation was too strong, a most disagreeable summation with the pain resulted; if too weak, there was but little effect on the pain.

With *electrical counterirritation*, the most effective strength, just below that which summated with the pain, caused twitching of the adjacent muscles. The sensation thus produced was not disagreeable, but a slight increase in current strength made it so.

The electric stimulus was applied as a series of single shocks, the frequency of which was varied between 1 and 300 per second. A slow frequency, such as one per second, was far less effective than more rapid rates and both subjects chose rates of 50 to 60 per second as the most effective. At rates above this the individual stimuli could hardly be distinguished and relief was less marked.

*Cold* stimuli were most effective when applied at a temperature of about 4 to 10° C. The ordinary ice bag felt too cold and was not so effective as more moderate temperatures.

The optimum temperature for *heat* counter-

irritation approximated  $40^{\circ}\text{C}$ .; this was effective in relieving the pain produced by subcutaneous injection of saline. But the pain produced by skin irritants, far from being relieved, was markedly intensified by all degrees of heat. This phenomenon has been described as characteristic of a stage of inflammation of the skin called by Sir Thomas Lewis (2) the "susceptible state," and the condition of the skin after the application of irritants in our experiments seemed identical with that described by him.

*Effect of counterirritation at different positions relative to the site of the painful stimulus.* As experience has already taught, the most effective position for counterirritation is over the site of the painful stimulus. However, when counterirritation was applied to the arm 6 inches proximal to the painful area, along the course of the nerve supplying it, considerable relief was secured. When it was applied to the arm distal to that area, relief was clearly detectable, but the effect on the pain was less. A similar statement can be made concerning strong counterirritation applied at remote points, such as the back or the other arm; slight but definite relief of pain in the arm followed. But more important than the degree of relief, the pattern was found to be similar to that shown in Figure 1, in which the counterirritant was applied over the site of the painful stimulus.

*Relative effects of different kinds of counterirritation.* When counterirritants were applied over the painful area produced by an injection of 10 per cent salt solution, both authors judged the relative effectiveness to be in the following descending order: cold, electric stimulation, heat, tactile stimulation, vibration, and the air jet. On the other hand, only cold gave effective relief from the pain caused by skin irritants.

*Interrupted counterirritation.* Inasmuch as there is a temporary relief of pain following the application of a counterirritant, and again after its removal, we concluded that the most effective means for the relief of pain would be the periodic application and withdrawal of a stimulus at an appropriate frequency. With electrical stimulation, the bombardment was switched on and off by means of contacts on a rotating sector operated on a synchronous clock motor. With heat and with cold, water bottles were applied and removed by

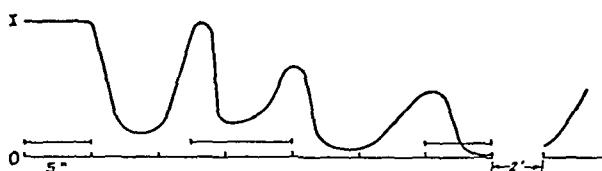


FIG. 2. EFFECT OF INTERRUPTED COUNTERIRRITATION BY COLD ON PAIN INDUCED BY 10 PER CENT NaCl

Time intervals 5 seconds with an interruption of 2 minutes. Bars indicate periods of application of cold bag.

the subject's partner. A similar effect was achieved by a lamp or a fan operated by a time switch. Figure 2 shows the sensory effects of such periodic stimulation.

There was no doubt in the authors' minds that by means of interrupted counterirritation far greater relief of pain could be attained than by a continuous application of counterirritation. Indeed in some cases severe discomfort could be relieved completely and continuously by a rhythm that approximated 5 seconds "on" and 10 seconds "off." In other instances, the relief could not be made complete, but the average level of pain was always markedly lowered by this method.

#### *Analysis of certain factors concerned in counterirritation*

*A. Experiments on ourselves.* When heat and cold are used for counterirritation, both stimulation of sensory nerve endings and changes in the caliber of adjacent blood vessels follow. We therefore designed experiments to ascertain whether the relief obtained was dependent on one or both of these factors.

In preliminary experiments, the effect of obstructing the circulation by an inflated cuff was studied. Figure 3 shows typical results. Inflation of the cuff to a pressure a little over the systolic promptly exaggerated the pain of skin irritation, and first diminished and then increased that from 10 per cent NaCl. Release of the cuff quickly decreased both types of pain. Venous obstruction produced much less effect. Inflation of the cuff to 30 mm. slowly augmented pain from capsicum and there was further increase on release. The same procedure had little effect on NaCl pain. These characteristic effects of circulatory obstruction (Figure 3) were not changed by immersion of the arm in hot ( $45^{\circ}\text{C}$ .) or cold

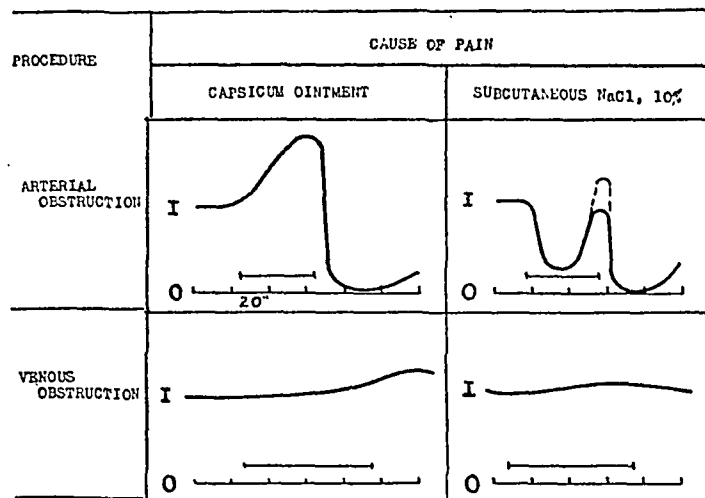


FIG. 3. EFFECT OF TOTAL OBSTRUCTION AND RELEASE OF THE CIRCULATION ON PAINS FROM SKIN IRRITANTS AND 10 PER CENT NaCl.

Effect of venous congestion by a cuff inflated to 30 mm. Hg. Obstruction of circulation indicated by bars. Time, 20 seconds.

(5° C.) baths during the experiments, so they were not dependent on temperature changes secondary to circulatory alteration.

If, after first inducing pain, the cuff was maintained at a pressure a little over the systolic for 5 minutes or longer, a plateau of painful sensation was secured which made tests of counterirritation possible. Under these conditions it was evident that heat, cold, and electrical stimulation had their usual effects on pain in spite of complete obstruction of the circulation. Venous congestion likewise caused no alteration of these effects.

In other experiments we induced pain by skin irritants and by 10 per cent NaCl in regions in which maximal vasodilatation had previously been induced by histamine or mecholyl iontophoresis, or by immersion of the arm in water at 45° C. for 20 minutes. We also produced pain in areas of vasoconstriction caused by adrenalin iontophoresis. Under all these conditions heat and cold produced their characteristic effects on the pain. We therefore concluded that the immediate action of these counterirritants in relieving pain was not dependent on any change in the circulation that they might produce.

In five experiments we measured skin temperature when applying heat and cold to either type of pain. In pain following 10 per cent NaCl the skin temperature bore no relation to the fluctuations in the pain when counterirritants were applied. In

the case of pain from capsicum the relation was closer, for heat increased it and cold decreased it. There were, however, temporary decreases in pain on removal of both heat and cold which had no counterpart on the skin temperature record.

*B. Animal experiments.* Our data indicated that part of the relief of counterirritation might be accomplished by an effect on the peripheral sensory nerves or their endings. Therefore, we turned to animal experiments in order to obtain records of action potentials of sensory nerves while we repeated on the animals the experiments which we had done on ourselves. In this way we sought to determine whether there was a peripheral basis for the sensations we had experienced in analogous experiments. It was not, however, possible to repeat the exact technique of all the human experiments in the animal preparations. The application of rubber bags containing hot or cold water set up tactile impulses which confused or obscured the response in the discharging pain fibers. In the case of heat the difficulty was overcome by using a radiant source.

With the assistance of Professor D. W. Bronk we experimented on a number of cats anesthetized with nembutal. Some hundreds of experiments were run on ten preparations. Cutaneous nerve twigs were freed and placed on electrodes leading to a suitable amplifier. Photographic records were made by means of a Matthews oscillograph. An area of skin on one limb was shaved and, after control records of the effects of counterirritants had been made, the shaved skin was rubbed with our irritant, capsolin ointment. This brought about a steady discharge of nerve impulses of high frequency. After this discharge had been established, the application to the treated skin of radiant heat of a degree insufficient to initiate a discharge from normal skin caused further exaggeration of the discharge (Figure 4a). In contrast to this, the application of cold by ice bags to the skin decreased or abolished the discharge (Figure 4b). Obstruction of the artery to the limb resulted in an increase in the discharge and on release this either diminished below the initial level or disappeared altogether for a few seconds (Figure 4c).

These responses in the cutaneous nerves followed in a general way the changes which would be expected if these nerve impulses caused the



Figure 4a

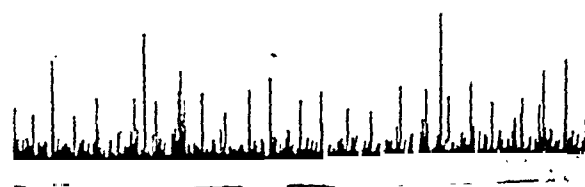


Figure 4b

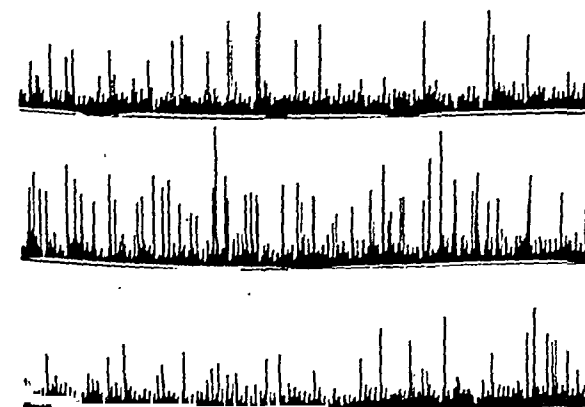


Figure 4c

modification of sensation which we observed under similar conditions in ourselves. For this reason it appears likely that the records we obtained were impulses from pain receptors. While we made no detailed analysis of the conduction velocities, the fibers were of the rapidly conducting type similar to those observed by Heinbecker and Bishop and others (3) to be responsible for pain.

The alterations in the sensory discharge in the animal preparations and in the sensations experienced by ourselves in similar experiments were comparable in a general way but not in all details. In the case of circulatory obstruction the parallelism was quite clear. It was also apparent in the case of the *application* of heat and cold, inasmuch as heat increased the peripheral discharge and cold diminished it. We did not, however, observe a temporary reduction in the sensory discharge on *removal* of the heat or cold, as one might have expected from the changes in sensation in similar experiments. For these temporary fluctuations in sensation some basis other than an altered peripheral sensory discharge must be sought.

#### Clinical experience

It is not to be expected that all the different types of pain encountered in the clinic will fall into the same pattern and be relieved by the same methods which have been successful in relieving the pain induced in our experiments. When experimenting on ourselves we worked with pains originating from a site at which counterirritation could be applied. In patients counterirritation must often be made at regions remote from the site of origin of the pain. Furthermore, we have demonstrated that some forms of counterirritation relieve one type of pain and increase another. In spite of these obstacles, our experience has given us a new point of view concerning relief of pain by counterirritation. We know well the great

dry de-  
FIG. 4. RECORD OF IMPULSES IN CUTANEOUS NERVES for pe-  
CATS

The nerves led from an area of skin treated by  
ointment. a; the top is a control; middle line, temporary re-  
radiant heat; lower, after removal of heat, shows the appli-  
control; lower, after application of cold. b due to a de-  
control; middle line, during occlusion of some central  
the part; lower line, on release of circulation. c, dry relief occurs  
one-fifth second; 4b, c, one second.





## DISCUSSION

In seeking an explanation for the differing effects of counterirritation upon pain from salt and from capsicum, it is necessary to consider the differences in origin of the pains. Capsolin causes pain by stimulating superficial cutaneous endings; sodium chloride, by stimulating nerve fibers as well as nerve endings lying deeper in the subcutaneous tissue. Furthermore, capsolin is applied to a large area of skin, while NaCl is injected into a small area, thereby making a spatial difference in the fibers and endings stimulated.

Since the capsolin-activated skin endings are quite superficial, their temperature can be rapidly altered by surface applications, and it is therefore *not surprising that the capsolin pain is easily affected by local temperature shifts*. Since the irritability of nervous tissue varies in the same direction as temperature change (4, 5), heat and cold applied over skin treated by capsolin increase or decrease the *peripheral sensory discharge*, as we found by direct observation, and thus increase or decrease pain.

On the other hand, surface applications could not readily alter the temperature of the deeper-lying nerves which are stimulated by subcutaneous NaCl. Consequently, it would be unlikely that sensation would fluctuate in direct relation to surface temperature. We indeed found no correlation between pain and skin temperature. Both heat and cold temporarily decrease NaCl pain. We must therefore look for some explanation other than an altered peripheral sensory discharge secondary to temperature change to account for these shifts in sensation in the case of NaCl pain.

Nor does a change in the peripheral discharge account for the temporary decrease in capsolin pain on removal of the heat or cold, for we found no modification at this time in the records of the nerve impulses.

We are thus led to consider the possibility that counterirritation may act by altering some central mechanism. Such a theory is not new; Cushny (6) suggested, on the basis of Head and McKenzie's data, that counterirritants act centrally. Several of our observations strengthen this possibility. Thus, while it is well known that the most effective site of counterirritation is over the painful area, nevertheless counterirritation applied

elsewhere is by no means ineffectual. Furthermore, we also found the typical pattern of relief on application and removal of the counterirritant, whether it was applied over the painful area or at some distant site unrelated in peripheral nerve supply.

A possible basis for a central sensory depression or inhibition is provided in recent experiments of Bronk (7). When a nerve cell which is stimulated chemically has its activity increased for a short time by electrical stimulation, there follows a period of diminished irritability during which the cell responds to the same chemical stimulus, either at a lower frequency or not at all.

If such a mechanism operates within the central nervous system it would explain our observation of the relief of pain on stopping counterirritation. The duration of the relief is of the same order of magnitude as Bronk's post-stimulatory depression, that is, a few seconds. It would account also for the fact that to obtain maximum relief the counterirritant must be of an intensity near that which augments pain, and therefore would explain the frequent increase in pain which we observed just preceding relief. For, as we saw above, to obtain Bronk's post-stimulatory inhibition, the activity of the nerve cells must be temporarily increased, and therefore the pain should be temporarily exaggerated. Finally, the identical effects of counterirritants which stimulate different types of specific skin endings—temperature, tactile, etc.—could be explained, provided the various fibers converged somewhere upon the cells activated by the painful stimulus. There is such a convergence within the optic thalamus, and thus it is possible that central depression could take place at this level.

To summarize: We can account for the effect of cold in relieving capsolin pain on the basis of a decreased peripheral sensory discharge due to lowering the temperature of pain endings. Our evidence suggests a central, post-stimulatory depression of the type described by Bronk for peripheral structures to explain the relief of pain on stopping counterirritation. The temporary relief of pain, which immediately follows the application of the counterirritant, is also due to a depression of sensation by alteration of some central mechanism, for a similar temporary relief occurs

(19) was followed for the gonadotropin. The urine was acidified with glacial acetic acid to the turning point of brom-cresol-green pH 3.8 to 5.4. Twelve to fourteen grams of tannic acid as a 20 per cent solution were then added and the precipitate was allowed to settle for half an hour in the ice chest. The precipitate was then collected by centrifugation, saving the supernatant, carefully washed with 95 per cent alcohol, three times with 80 per cent alcohol, left overnight in 95 per cent alcohol and then dried with acetone and ether. The washings were pooled to be treated as described below. The dried precipitate was extracted three times with slightly alkaline water, then neutralized and brought to a volume of 24 cc. per forty-eight hours' collection immediately before the assay. The dried tannate powder is stable for long periods without deterioration but the solution deteriorates slowly.

The pooled alcohol, acetone and ether washings of the tannic acid precipitate were combined with the chloroform preservative of the original urine. The volatile solvents were then removed over a water bath temperature of 90 to 100° C. under negative pressure. The watery fraction was added to the supernatant from the original tannic acid precipitate. This mixture was acidified with concentrated HCl (about 12N) to pH 1 to 2, the turning point of orange IV, and 3.3 cc. concentrated HCl per 100 cc. original urine were added to insure excess acid (22). The whole was boiled under a reflux condenser for half an hour, a time insuring maximum recovery of both estrogen and androgen (23, 24, 25). Acid hydrolysis split the combined estrogen and androgen, a necessary step before assay could be conducted.<sup>2</sup> The substance thus freed and the small fraction of the hormones in the urine not combined originally are soluble in ether. Accordingly, the hydrolysate was then cooled and extracted with ether in a four liter pyrex separatory funnel, using 200 cc. ether per liter of original urine for the first extraction, then three times more with 100 cc. per liter, rinsing with the ether the flask in which the urine was hydrolyzed. No emulsion formed during any of the extractions with the ether. The pooled ether extracts were then washed three times with 30 cc. H<sub>2</sub>O each per liter of original urine.

The estrogenic fraction was then separated from the androgenic fraction by extracting the ether three times with 100 cc. each of 1 N NaOH per liter of original urine. The remaining ether, containing the androgen, was washed three times with water as before, and the washings were added to the NaOH extract containing the estrogen. The NaOH fraction was acidified to pH 4.5 to 5, the turning point of congo red, with concentrated HCl, and extracted immediately three times with 100 cc. of ether per liter of original urine each time. The ether, now containing the estrogen, was washed three times with water as above. The ether fractions of estrogen

and androgen were placed in the ice box overnight to allow the water to drain from the walls of the separatory funnel, and the water was then removed. The androgenic ether fraction was evaporated to dryness under negative pressure on the water bath and the residue redissolved in 10 cc. of absolute alcohol per twenty-four hours of original collection when ready for assay. This fraction permitted colorimetric or biologic assay to be done. The estrogenic ether fraction was evaporated to a small volume, transferred to a small Erlenmeyer flask, extracted with half the desired volume of corn oil (Mazola), and the ether removed by heating over a water bath. The tarry residue was redissolved in a small volume of ether and re-extracted with oil and the ether removed as before. The oils were combined, a total of 12 cc. of oil being used per forty-eight hours' collection.

*Assay.* All assays have been conducted on six to eighteen animals, twelve being used for most of the specimens. Hypophyseal gonadotropin was assayed by the mouse uterine weight method of Levin and Tyndale (20), three mice per dose level being used. Unit levels of 5, 10, 20, 27, 40, 60 and 78 units per twenty-four hours were tested for, as necessary. A uterine weight of 11.0 mgm. or over in two of the three mice was considered positive if the next higher unit level was negative and the next lower unit level positive. No serious discrepancies have been noted between responses at these different dose levels. Toxicity of the extract was high at the time of the menses. Reprecipitation in acetone at this time generally permitted assay of 5 units per twenty-four hours in those instances where the animals were killed by the original extract. Ten units could practically always be assayed without reprecipitation.

Estrogen was assayed by the Kahnt-Doisy (4) procedure, using the castrated rat vaginal smear. The method was followed in exact detail. Three rats per dose level were used as above for the gonadotropic assay and the same unit levels tested. The rats have been repeatedly tested for sensitivity to crystalline estrone.<sup>3</sup> One rat unit has been found to be equivalent to 0.7 to 0.8 gamma of crystalline estrone.

Androgen, or 17-keto-steroid, as it will hereafter be referred to in this paper, was determined by the colorimetric reaction of Zimmermann (16), as detailed by Callow and Callow (26), except that aqueous KOH 2.45 N was substituted for alcoholic KOH (27). Every run was controlled by concurrent determinations on 5, 10, 15 and 20 mgm. of crystalline androsterone.<sup>3</sup> This was necessitated by the range of color produced in various tests for a given dose of standard material, probably caused by slight variation in the reagents. Since the mother liquor from the urine was colored, a blank of this solution was run as for the test but alcohol was substituted for m-dinitrobenzene. The value then obtained was subtracted from the total value with m-

<sup>2</sup> Talbot, J. Biol. Chem., 1940, 136, 365, states that hydrolysis destroys 30 per cent of dehydroisoandrosterone. This compound is a small fraction of the normal keto-steroids. These are not similarly affected.

<sup>3</sup> The author is indebted to Dr. Edward Henderson of the Schering Corporation, Bloomfield, N. J., for the generous supply of estrone and androsterone which was used.

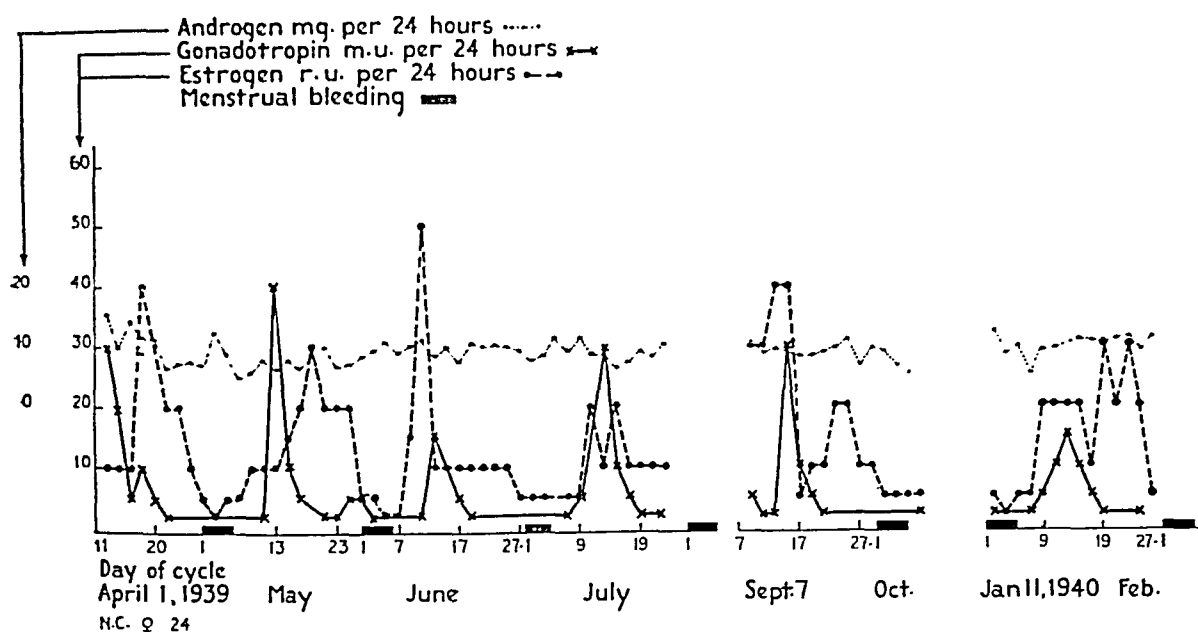


FIG. 1. FORTY-EIGHT-HOUR EXCRETION OF 17-KETO-STEROID, ESTROGEN, AND GONADOTROPIN IN RELATION TO THE MENSTRUAL CYCLE, INCLUDING THREE SINGLE CYCLES AT INTERVALS THROUGHOUT THE YEAR. SUBJECT N. C.

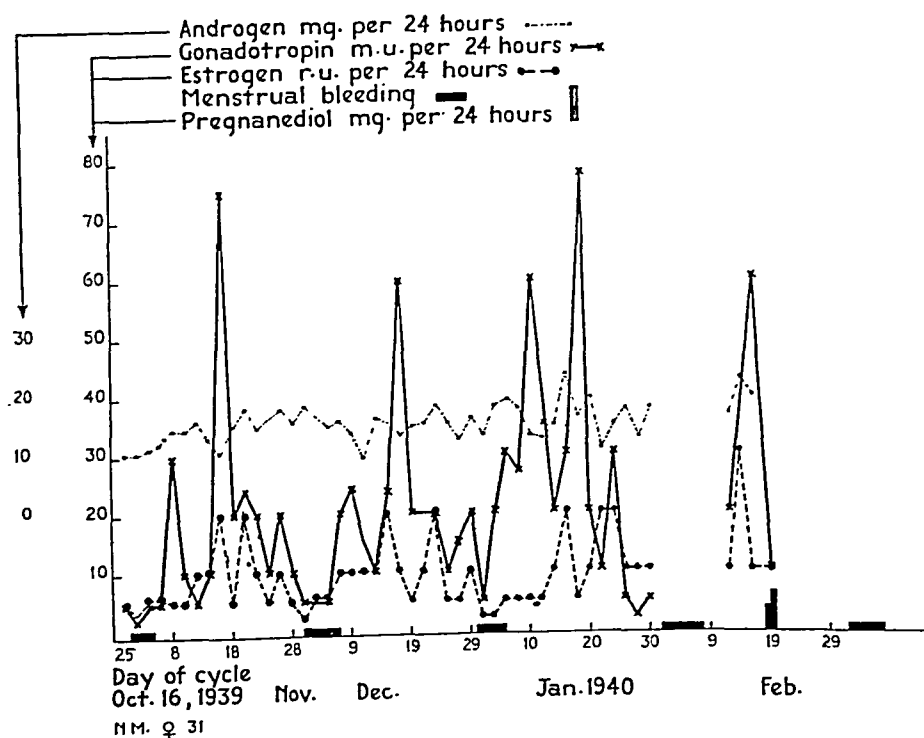


FIG. 2. FORTY-EIGHT-HOUR EXCRETION OF 17-KETO-STEROID, ESTROGEN, AND GONADOTROPIN IN RELATION TO THE MENSTRUAL CYCLE, INCLUDING PREGNANEDIOL GLUCURONIDATE EXCRETION IN THE MID-PERIOD OF AN ADDITIONAL CYCLE. SUBJECT N. M.

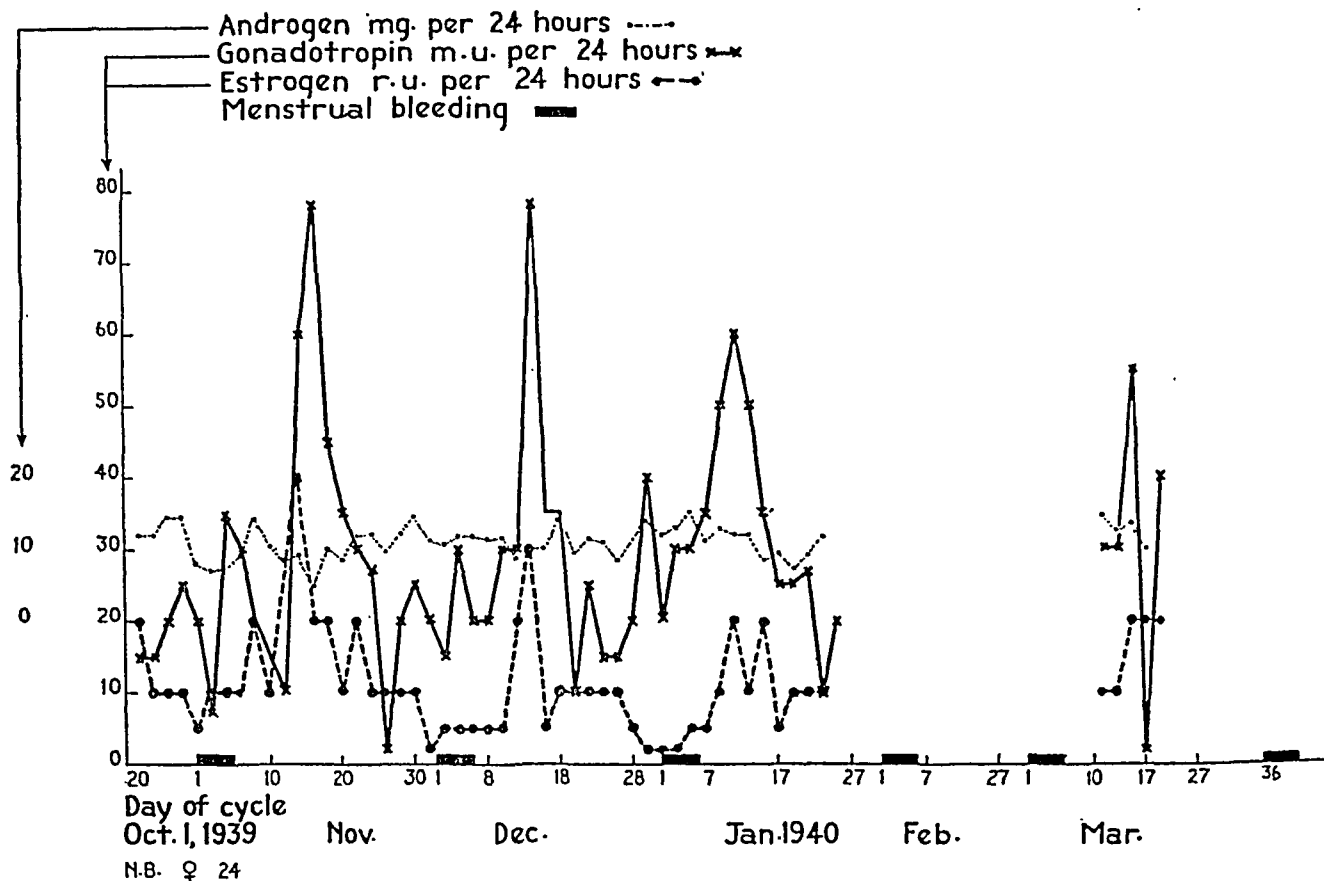


FIG. 3. FORTY-EIGHT-HOUR EXCRETION OF 17-KETO-STEROID, ESTROGEN, AND GONADOTROPIN IN RELATION TO THE MENSTRUAL CYCLE, WITH AN ADDITIONAL MID-CYCLE ILLUSTRATING FAILURE OF PREGNANEDIOL GLUCURONIDATE TO BE EXCRETED BY TWENTIETH DAY IN A THIRTY-SIX-DAY CYCLE. SUBJECT N. B.

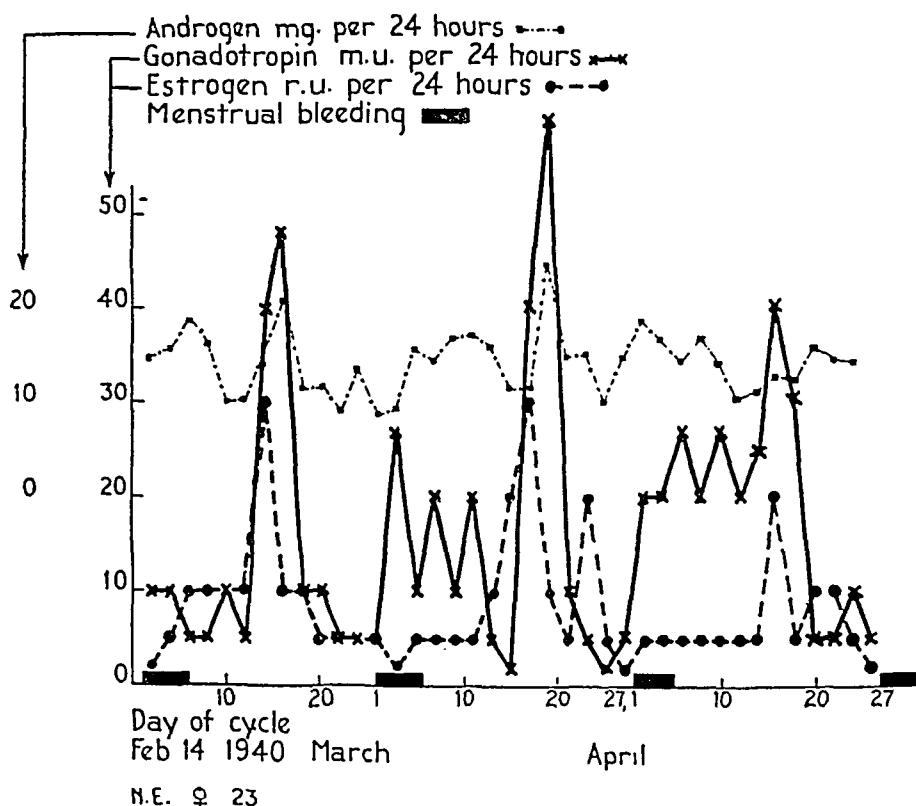


FIG. 4. FORTY-EIGHT-HOUR EXCRETION OF 17-KETO-STEROID, ESTROGEN, AND GONADOTROPIN IN RELATION TO THE MENSTRUAL CYCLE, AND ILLUSTRATING FAILURE OF PREGNANEDIOL TO BE EXCRETED THROUGHOUT ENTIRE LAST CYCLE. SUBJECT N. E.

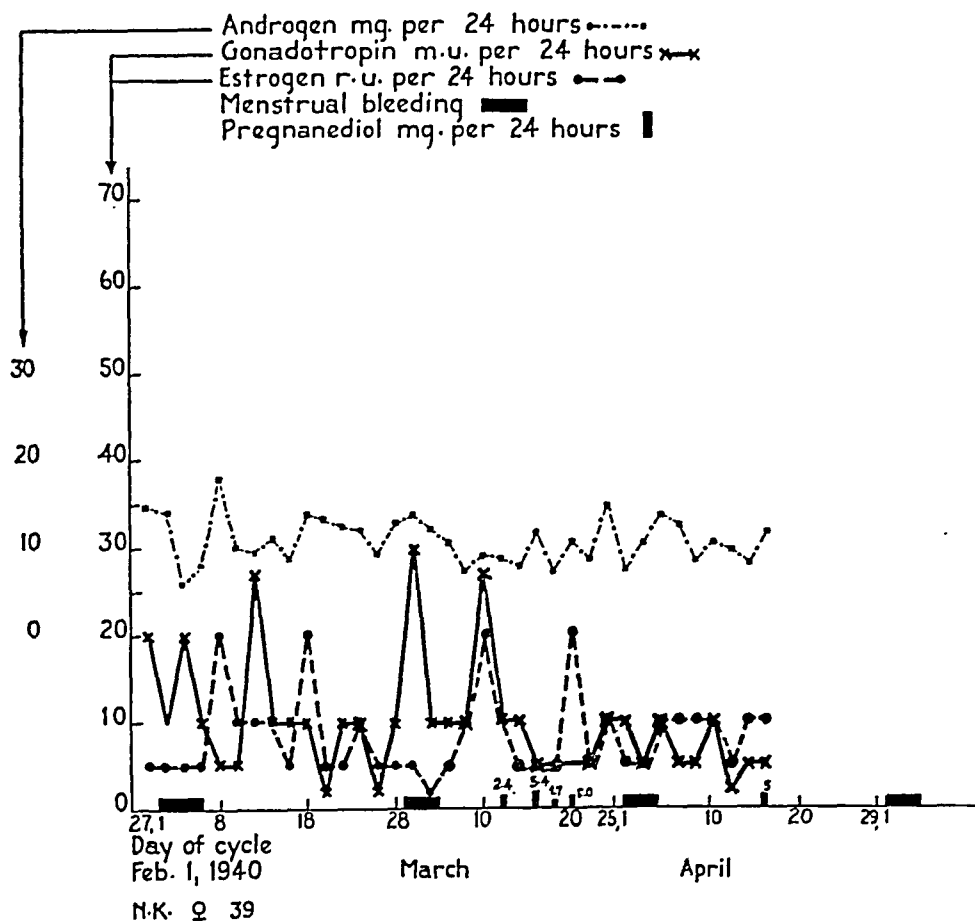


FIG. 5. FORTY-EIGHT-HOUR EXCRETION OF 17-KETO-STEROID, ESTROGEN, AND GONADOTROPIN IN RELATION TO THE MENSTRUAL CYCLE, AND ILLUSTRATING FAILURE OF PEAKS OF GONADOTROPIN AND ESTROGEN TO BE DEMONSTRATED IN LAST MENSTRUAL CYCLE, AND SHOWING APPEARANCE OF PREGNANEDIOL GLUCURONIDATE IN THE ABSENCE OF THESE PEAKS. SUBJECT N. K.

dinitrobenzene. A reagent blank was also run. Colorimetric determinations were read on a neutral wedge photometer (American Instrument Company), using two 0.999 inch cells, one containing the blank in one beam and the solution to be read in the other. Readings were taken with a filter having a centrum at 510 millimicrons. Values for the 17-keto-steroid content of the unknown are expressed in mgm. equivalents of androsterone per twenty-four hours.

*Animals.* A colony of mice and rats was maintained solely for the assays. The mice were of the Klasek or Swiss strain. Rats were the Long-Evans strain. The mice were fed on a standard mouse biscuit, with water *ad lib*. The rats were given a standard diet, McCollum mixture number 1 with water *ad lib*. and lettuce supplements. Weaning weight for the mice was 8 to 13 grams at twenty-two days, averaging 10 to 11 grams. Only twenty-two to twenty-three-day old mice were used. The rats were castrated at four months of age and tested for responsivity according to the method of Kahnt and Doisy.

*Extracts.* Tannate and estrogenic extracts were assayed immediately after preparation in almost every instance. The androgens were kept dry in stoppered flasks for one to two weeks, and then dissolved in alcohol on the day of the determination. All extracts were kept in the ice box when not being used.

#### EXPERIMENTAL

The urine which was assayed was collected from five healthy, normal women with regular menstrual cycles. The subjects carried on their daily routine during the course of the experiment. Their ages were: twenty-three, twenty-four (2 subjects), thirty-one and thirty-nine. Onset of menses occurred at ten and one-half to thirteen years of age. There were no menstrual abnormalities beyond slight dysmenorrhea the first day of bleeding. Menses were moderate in flow except for the first

two days of N. M.'s cycle when the flow was profuse. The patients were healthy throughout the study. Subjects N. K. and N. B. were married.

The output of hormones for each of the five subjects (N. C., N. M., N. B., N. E., and N. K.) is shown in Figures 1 to 5. The general pattern of all cycles except the last one of N. K. is similar in that there is a more or less constant 17-keto-steroid excretion, a central peak of gonadotropic and estrogen excretion, followed by a second peak of estrogen output. However, the pattern is not exactly reduplicated from cycle to cycle in the same individual or from subject to subject. The relationship between the peaks of estrogen and gonadotropin is not constant (Table I). The initial estrogen peak may precede, occur with, or come later than the gonadotropin peak. The time in the cycle that both these peaks occur is also variable, as is the actual length of the menstrual cycles. The peaks, however, tend to occur later or earlier as the cycle is longer or shorter (N. M., N. B., N. K.). The 17-keto-steroid level (Table II) fluctuates from day to day within relatively narrow limits for the individual, but varies between subjects.

TABLE I

*The days of occurrence in the menstrual cycle of the peaks of gonadotropin and estrogen excretion and of first appearance of pregnanediol glucuronide*

Subject	Number of cycle	Duration of cycle	Peak of gonadotropin	Peak of estrogen	First appearance of pregnanediol
N.C.	1	27	11 & 12	17 & 18	No determinations
	2	26	12 & 13	18 & 19	
	3	27	12 & 13	10 & 11	
	4	27	13 & 14	11 & 12	
	5	29	13 & 14	11-14	
	6	27	13 & 14	9-12	
N.M.	1	29	15 & 16	15 & 16	18 & 19
	2	29	16 & 17	14 & 15	
	3	30	17 & 18	15 & 16	
	4	32	15 & 16	13 & 14	
N.B.	1	32	16 & 17	14 & 15	None
	2	31	14 & 15	14 & 15	
	3	31	11 & 12	11 & 12	
	4	36	14 & 15 20 & 21?	14 & 15?	
N.E.	1	25	15 & 16	13 & 14	None
	2	26	18 & 19	16 & 17	
	3	28	16 & 17	16 & 17	
N.K.	1	28	11 & 12	7 & 8	12 & 13
	2	25	1 & 2 10 & 11	10 & 11	
	3	27	None	None	17 & 18

TABLE II

*Range of normal values for the excretion of androgen as 17-keto-steroid, estrogen and gonadotropin, excluding a few extreme values*

Subject	Androgen 17-keto-steroid  mgm. per 24 hours	Estrogen  rat units per 24 hours	Gonadotropin  mouse units per 24 hours
N.C. Average	5.4-12.5 10.0	<5-50	<5-40
N.M. Average	10.6-19.6 15.0	<5-30	<5-78
N.B. Average	7.4-14.6 10.5	<5-40	<5-78
N.E. Average	8.9-18.9 14.0	<5-30	<5-60
N.K. Average	5.7-14.7 11.0	<5-20	<5-30
Range for series	5.4-19.6	<5-50	<5-78

*Gonadotropin.* Contrary to previous work, gonadotropin was demonstrable throughout the cycles of all but one subject (N. C.). In this latter, less than 5 mouse units per twenty-four hours of gonadotropin were excreted, except during the middle of the cycle from the ninth to about the sixteenth day. The relatively constant amounts of gonadotropin throughout the cycles of the other subjects were also replaced by a sharp rise in output about the middle of the cycle. This peak of gonadotropin output reached its maximum at various times in the cycle, however, even in the same individual, varying from the tenth to the eighteenth day of the cycle in the series, or as with N. B., from the eleventh to possibly the twentieth day when the menses was delayed. The height of the peak varied from 15 to 80 mouse units per twenty-four hours but, except for N. K.'s last cycle, it was relatively constant for the individual. The peak of gonadotropin was sometimes preceded by a lesser peak. Thus, in one cycle each of subjects N. M. and N. B. there was a preliminary rise in gonadotropin output to 60 units. This preliminary peak was not associated with a corresponding estrogen peak, but was followed later by a sharper gonadotropin rise associated with a peak of estrogen. Gonadotropin may be present in large amounts during menstrual bleeding (N. M., N. B., N. K.). There is a range in excretion of gonadotropin for this series

of from less than 5 mouse units to 78 mouse units per twenty-four hours. This broad range has been found in a single individual's output, depending on the time of the cycle.

*Estrogen.* Estrogen values represent the total of free and combined estrogen. No attempt has been made to separate the various estrogens as reported by Smith, Smith and Pincus (28). The double peak of excretion described by Gustavson *et al.* (29) was generally found in this series. However, the contour of the excretion pattern varied considerably, a double peak being absent (N. C., N. K.) or hard to differentiate (N. C.). Estrogen was present throughout the cycle except about the time of menstrual bleeding, though its disappearance has no constant relation to the time of onset of bleeding (N. C., etc.). Values of excretion varied considerably from cycle to cycle. Thus, less than 5 rat units per twenty-four hours were found for forty-eight hours at one point during the menses, although rarely for a longer time (N. B.). At other times, values from 5 rat units to 50 rat units per twenty-four hours have been found depending on the time of the cycle. There seems to be a tendency to a slow rise and fall in the amount of hormone excreted after and before the menses, with a spurt in excretion during the mid-cycle (N. C., etc.) and again after a week or so. The point at which these peaks of estrogen occur is quite variable. The time of appearance of the first peak of excretion may vary from cycle to cycle in a given individual—for example, from day 9 to day 18 in N. C. or from day 7 to day 18 in the series. The height of the peak, too, may vary from 20 to 50 rat units per twenty-four hours.

*Androgen or 17-keto-steroid.* The androgen excretion as determined colorimetrically is really a measure of 17-keto-steroid excretion (26). The ketonic fraction was not separated from the non-ketonic fraction (30) in this study. The error resulting is probably not significant in normal urine. It should be mentioned that the introduction of Aq. KOH instead of alcoholic KOH may be responsible for altered reactivity of the various keto-steroids, although the total values obtained for this series compare favorably with those obtained with alcoholic KOH.

A fairly constant mean level of 17-keto-steroid excretion for a given individual throughout the

cycle was found. There was a day-to-day range of plus or minus 5 mgm., with an occasional more extreme value. The level of output varied from subject to subject but did not seem to show seasonal variation (N. C.). Normal values of excretion ranged from 5.4 to 19.6 mgm. if a few extremes were excluded.

*Pregnanediol.* Through the courtesy of Dr. E. T. Engle pregnanediol determinations were made during the cycles of all but N. C. (Table I) according to the method of Venning and Browne (18). In the case of N. M. and the first cycle of N. K. pregnanediol was present forty-eight to seventy-two hours after the peaks of estrogen and gonadotropic excretion. This is harmonious with the reports of Venning and Browne (18) that pregnanediol appeared shortly after the one point in the cycle at which they were able to demonstrate gonadotropin. However, in this series, no pregnanediol was demonstrable throughout the entire last cycle of N. E. and up to the nineteenth day in the last cycle of N. B. It should be mentioned, though, that the mensis was unusually late in appearing in this latter cycle. Pregnanediol appeared without an associated peak of gonadotropin or estrogen in the third cycle of N. K.

#### DISCUSSION

Data have been obtained concerning the quantitative excretion of gonadotropin, estrogen and 17-keto-steroid through eighteen cycles in five medically normal women. Except in one subject, these hormones were found throughout the entire menstrual cycle. There is, however, a cyclic influence on the excretion of gonadotropin and estrogen. A sudden peak of excretion of both hormones was generally found near the mid-interval with a reduplication of the estrogen peak later in the cycle. The 17-keto-steroid output, on the other hand, varied from day to day but showed no cyclic change. This relative constancy of output of 17-keto-steroid both for the individual and between individuals makes it possible to define the limits of normal excretion. Outside this range abnormality would be suspected.

The variation in pattern of excretion of gonadotropin and estrogen in different cycles makes the determination of the range of normality much more difficult. Thus the excretion curves of the subject N. K. are extremely variable, the last



cycle showing no peaks at the mid-interval. Had this cycle been assayed in a patient with some abnormality, this cycle might well have been interpreted as the result of a pathological state. The relatively normal contour of the remaining cycles, however, suggests the error of such an interpretation, although the possibility exists that there was some temporary abnormality. If the assay of the urinary output of an entire menstrual cycle may be inadequate, the significance of single twenty-four- or forty-eight-hour specimens is even more open to question. Single assays for estrogen and gonadotropin, as seen from this study, would be indicative of abnormality only if extremely high values were found, such as are found after castration and the menopause in the case of the gonadotropin. Very low values of less than 5 units per twenty-four hours may be significant also although, as seen in the case of subject N. C., extremely low values may be meaningless if found in only one specimen. However, the repeated finding of very low values, at weekly intervals for example, would have significance in view of the normally cyclic character of excretion.

The appearance of peaks of excretion of estrogen and gonadotropin near the middle of the menstrual cycle raises the speculation as to whether these peaks are related to ovulation. Kurzrok (31), using a less sensitive method of assay, obtained a positive reaction for gonadotropin only for one day in the cycle and interpreted this as indicating ovulation. Venning and Browne later pointed out that pregnanediol appeared shortly after this peak, indicating an association with corpus luteum formation. D'Amour (32) found several days in the cycle on which gonadotropin was present and so postulated several ovulations in a cycle.

The present study reveals that gonadotropin and estrogen are generally excreted throughout the cycle but that there is only one definite and associated peak of excretion of both hormones near the middle of the cycle. This peak is undoubtedly the point at which the gonadotropin was found in the earlier studies. The gonadotropin peak may be reduplicated (subjects N. M. and N. B.) but there is still only one time in the cycle when the gonadotropin peak is associated with the estrogen peak, a point also noted in the cycles recently reported by Von Haam and Rothermich (33).

This persistent relation at about the middle third of the cycle suggests that the phenomenon is associated with ovulation and, if so, indicates that there has been only one ovulation per cycle in these subjects. Further support for this view is seen in the appearance of pregnanediol shortly after this point in each of two subjects (N. M. and N. K.), revealing that a functional corpus luteum was formed about this time and implying that ovulation probably occurred as well. On the other hand, corpus luteum formation may be shown by the appearance of pregnanediol in the absence of the gonadotropin-estrogen peaks (last cycle of N. K.) and the latter peaks may occur without the subsequent excretion of pregnanediol (last cycle of N. E.). Thus the relation of the peaks of gonadotropin-estrogen excretion to ovulation remains presumptive pending further study.

The almost constant occurrence of the peaks of estrogen and gonadotropin output suggests a relationship between the two. However, the estrogen peak may appear before, with, or after the gonadotropin peak, and the shifting time relations involved make it difficult to explain either peak as the result of the other, as advanced by D'Amour (34). It would appear, rather, that the peaks may reflect two concurrent rhythms. One rhythm is the development of the ovarian follicle, reflected by the estrogen excretion, which takes place in the presence of the relatively constant output of hypophyseal gonadotropin that is found during the first part of the cycle preceding the peak of gonadotropin excretion. The other rhythm is the independent, though somehow related, one occurring in the anterior hypophysis. This results in a sudden release of an excess of gonadotropin at some time during the middle third of the cycle. As stated above, the association between the gonadotropin-estrogen peaks and the occurrence of ovulation has not been established. However, if this relationship should be demonstrated, it would appear likely that the gonadotropin peak results in ovulation if the gonadotropin spurt occurs at a time when the ovarian follicle has reached maturity, and does not produce ovulation if the follicle has not yet matured. Evidence for this supposition is found in the cycles of N. M. and N. B. in which early gonadotropin peaks, occurring before the estrogen peak, were reduplicated later at the time of the rise in estrogen excretion.

The mechanism coordinating the two rhythms is not clear.

There is one possibility which must be considered in interpreting any peak of hormonal output in the urine—the possibility of a temporary change in renal permeability which would permit excess excretion of the hormones. In this instance, the peak would be only an apparent one and not a true reflection of the rate of secretion of the hormones. Frank (35) found no evidence for such a change in permeability. Also, under such circumstances, one would expect the androgen output to rise cyclically in the same specimens in which the titer of gonadotropin and estrogen is rising.

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# EFFECT OF VARYING INTAKE OF PROTEIN AND SALTS ON THE COMPOSITION AND SPECIFIC GRAVITY OF URINE<sup>1</sup>

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It has been shown in a previous paper (1) that the specific gravity of urine is a simple additive function of the concentrations of the individual solutes. By the use of appropriate factors, the specific gravity "pattern," i.e., the fraction of the specific gravity accounted for by each of the constituents of the urine, may be calculated when the composition is known. In urine from subjects on a standard 40 or 50 gram protein diet, urea accounted for 15 to 20 per cent, chloride for 25 to 30 per cent, sulfate and phosphate together for 15 to 25 per cent, bicarbonate for 1 to 5 per cent, and creatinine for 1 to 2 per cent of the observed specific gravity. On a 100 or 110 gram protein diet, urea, sulfate, and phosphate made a slightly greater contribution to the specific gravity. On the lower protein diet, 70 to 80 per cent, and on the higher protein diet, 80 to 90 per cent of the specific gravity could be accounted for by the substances determined (urea, creatinine, inorganic salts, and ammonia). The undetermined fraction of specific gravity made up 10 to 30 per cent of the observed value and, in the same urines, the excretion of undetermined solids amounted to 9 to 15 grams daily, which was 10 to 30 per cent of the total solids. The undetermined solids were organic substances, about one-half being organic acids, and the daily excretion increased only slightly when the protein intake was more than doubled. The specific gravity pattern observed in this previous study was remarkably constant, presumably attributable to the fact that the subjects were on relatively uniform diets, particularly with regard to protein and salt intake.

The present study is an extension of this investigation to include the effect of free choice of diet, the influence of specific dietary factors, and the ability of the kidney to vary the composition and specific gravity pattern of urine under ex-

perimentally produced changes in the internal environment.

All urine specimens were collected and analyzed as described in the previous paper (1). In cases where the collection period was less than 24 hours, or where the subject was held to a controlled regime, the details are given in the discussion. Sodium and potassium were determined in all cases and the specific gravity contributions of the inorganic constituents were calculated by Method B, previously described.

For analysis of serum the following methods were used: urea, Van Slyke hypobromite; total protein, micro Kjeldahl; chloride, Van Slyke and Sendroy; phosphate, Fiske and Subbarow (2); creatinine, Rehberg (3); and sulfate, Power and Wakefield (4). Cell volume was determined on the whole blood.

The excretion of water has no effect on the specific gravity pattern of urine when other conditions are kept constant. This was shown in the previous paper where subjects on a controlled diet excreted urines of specific gravity ranging from 1.003 to 1.028 with practically identical patterns.

Since the kidney is not the chief route for excretion of the end products of carbohydrate metabolism, the effect of varying the intake of carbohydrate has not been studied. In all diets, however, sufficient carbohydrate was included to maintain normal fat metabolism and insure adequate caloric intake.

Similarly, the products of normal fat metabolism are not excreted to any great extent in the urine. This was tested in one experiment, as shown in Table I, where fat intake was reduced one-half, protein, carbohydrate, and salt intake remaining constant. On the second day of the low fat intake, a 24-hour specimen was collected. No significant change in the composition or specific gravity pattern was found. Protein intake, on the other hand, is one of the principal factors con-

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TABLE I

*Effect of protein and fat on the specific gravity pattern of urine*

Date	Diet			Urine volume	Specific gravity	pH	Nitrogen output	Blood urea nitrogen	Output undetermined solids	Per cent of specific gravity accounted for by							
	Protein	Fat	Carbohydrate							Urea	Creatinine	Chloride	SO <sub>4</sub>	H <sub>2</sub> PO <sub>4</sub>	HPO <sub>4</sub>	HCO <sub>3</sub>	Total
	grams	grams	grams	cc.	20°/4°		grams	mgm. per 100 cc.	grams								
October 30, 1939	19	114	350	1682	1.0080	7.00	4.5	4.0	9.9	11.7	2.0	41.6	6.9	3.1	6.8	7.2	79.3
November 22, 1939	57	114	400	1685	1.0126	7.30	7.9	10.7	11.6	14.5	1.5	33.8	7.3	1.9	8.8	10.4	78.2
February 7, 1940	95	89	250	1818	1.0156	6.59	14.1	12.7	12.5	21.4	1.2	34.4	11.7	5.5	4.7	2.0	80.9
April 24, 1940	150	110	170	1788	1.0167	5.70	21.9	17.4	17.2	33.4	1.2	17.3	17.4	10.1	1.0		80.4
April 26, 1940	150	60	170	1728	1.0167	5.52	22.7	17.4	16.9	35.1	1.3	18.0	17.5	11.8	0.8		84.5

trolling urine composition and specific gravity pattern, as has been shown in previously presented data. Table I gives data from Subject L. whose protein intake was varied over a greater range, from 19 to 150 grams daily.<sup>2</sup> In these experiments, the subject remained on each diet until nitrogen excretion indicated equilibrium, and a 24-hour urine specimen was then collected for analysis. Fluid intake was kept constant during all of the urine collection periods.

When salt intake is kept relatively constant, there is a striking correlation between urea or total nitrogen output and the fraction of specific gravity of the urine accounted for by urea. On a 19 gram protein intake, urea accounted for 11.7 per cent, while on a 150 gram intake, this rose to 33.4 per cent. The contribution of sulfate to the specific gravity also increased and the ratio of the contributions of sulfate to urea is constant, varying only between 0.50 to 0.60. Since the sulphur to nitrogen ratio of urine is essentially constant, this result is to be expected. The phosphate contribution to specific gravity increases only slightly, the increase in phosphorus output being balanced by the shift from  $\text{HPO}_4^{2-}$  to  $\text{H}_2\text{PO}_4^-$  which occurs with increased protein metabolism and fall in pH. Bicarbonate plays a significant rôle in the specific gravity pattern on the two lower protein diets be-

cause of the higher pH values but becomes negligible with greater protein intake. Chloride, since its output is relatively constant, contributes a smaller proportion of the specific gravity as the output of other substances is increased. Creatinine behaves in a similar manner.

The excretion of undetermined solids also varies with protein metabolism, approximately doubling with a five-fold increase in nitrogen output. The increase in the output of undetermined solids with increase in protein metabolism was previously noted but not considered to be definitely significant when 40 or 50 gram and 100 or 110 gram protein diets were compared. The greater protein range covered in these experiments, however, indicates a direct relationship. If the outputs of total solids (grams) and organic acids (milliequivalents) are correlated, a constant value is obtained so that organic acids seem to account for a constant fraction of the undetermined solids, regardless of the diet. The remainder is due to other compounds, the output of which also increases with increased protein metabolism. Change in the fat content of the diet has no effect on the undetermined solids.

The effect of salts on the specific gravity pattern was studied from several aspects: (1) The addition of 10 grams of sodium chloride to the diet, keeping other dietary factors constant, both on 50 gram and 110 gram protein diets (M.); (2) salt depletion produced by removal of salt from the diet coupled with sweating (Z.), by pyloric obstruction (C.), or by minimal salt ingestion with minimal (600 calories) dietary intake (G. M.); (3) intravenous injection of concentrated solutions of various salts after a period of water deprivation.

The results of the first two studies are sum-

<sup>2</sup> In this and following tables, data on concentration and output of the urine constituents are not given. Sufficient data are included, however, to make possible the calculation of the concentration or output of urea and creatinine (in millimols or grams) and of the inorganic anions (in milliequivalents) when used in conjunction with the specific gravity factors in Table I of the previous paper (1). A close approximation of the output of salts may be obtained by considering the cation to be sodium alone.

TABLE II

*Effect of sodium chloride on the specific gravity pattern of urine*

Subject	Date	Conditions	Urine vol- ume	Spe- cific gravity	pH	Per cent of specific gravity due to:										Output Cl as NaCl	Output unde- ter- mined solids
						Urea	Creat- inine	Chlo- ride	SO <sub>4</sub>	H <sub>2</sub> PO <sub>4</sub>	HPO <sub>4</sub>	HCO <sub>3</sub>	Total				
M.	October 4, 1939	50 grams protein—control	cc.	207/4*													
	October 12, 1939	50 grams protein—10 grams salt added	580 995	1.0265 1.0212	6.4 6.6	20.3 18.5	2.3 1.6	27.7 38.6	10.2 6.5	8.3 5.9	4.6 5.4	0.9 0.9	74.3 77.4	6.4 14.0			
M.	October 30, 1939	110 grams protein—control	1010	1.0261	5.9	31.3	1.8	24.7	14.2	11.0	1.8		84.8	9.8			
	October 31, 1939	110 grams protein—10 grams salt added	1220	1.0264	5.7	24.8	1.5	37.4	10.8	9.8	1.0		85.3	18.1			
Z.	February 27, 1940	12-hour specimen—control	226	1.0261	6.01	18.3	3.1	27.1	10.3	12.5	2.5		73.8	2.4	4.5		
	March 4, 1940	12-hour specimen—control	206	1.0282	6.30	15.2	2.9	25.2	10.6	12.1	5.1	0.6	71.7	2.2	4.9		
	May 1, 1940	12-hour specimen—salt depletion	166	1.0296	5.44	25.0	3.7	0.2	17.4	23.6	1.4		71.3	0.02	4.6		
G.M.	April 15, 1940	Low protein and salt	250	1.0200	6.18	25.7	3.5	3.7	6.6	7.7	2.0		49.2	0.44	7.4		
C.	June 18, 1940	Pyloric obstruction	810	1.0227	6.40	34.1	1.5	0.1	16.1	11.4	5.8	0.4	69.4	0.02	18.5		
	June 28, 1940	After salt therapy	1500	1.0097	7.00	19.0	1.1	33.0	8.1	5.2	11.8	2.6	80.8	5.4	8.9		

marized in Table II. When 10 grams of sodium chloride are added to a diet already containing 6 to 10 grams (M.), the excess is largely excreted within 24 hours with a moderate increase in water excretion (fluid intake was constant for each dietary regime). There is an increase in the chloride fraction of specific gravity from 28 per cent to 39 per cent on the low protein and from 25 per cent to 37 per cent on the high protein diet. The specific gravity contributions of the other constituents are reduced sufficiently so that the total specific gravity accounted for is approximately the same. This reduction is the result of dilution, since the total output of the substance other than chloride was unchanged. This would indicate that the undetermined solids are diluted in the same proportion, although data on this point were not obtained in these experiments with Subject M. The data do show, however, that salt intake, which is quite variable among individuals, plays a very important rôle in fixing the specific gravity pattern.

The effect of salt deprivation on the specific gravity pattern of the urine is shown by Subject Z. The urines analyzed in this study are overnight specimens representing the final 12 hours of a 33-hour concentration test. On a control diet of 50 grams of protein and a salt intake of approximately 6 grams, the 12-hour urine specimens show the usual picture found with 24-hour specimens. The subject was then placed on a low salt diet containing 0.5 to 1.0 gram daily and an attempt was made to produce salt depletion by sweating, using the technique described by McCance (5). The

average daily loss of chloride in the sweat was 2.5 grams as sodium chloride, thus placing the subject in negative salt balance. The chloride content of the urine fell rapidly, finally reaching a 24-hour output level of less than 0.1 gram, but the serum chloride did not fall lower than 95.6 milliequivalents per liter. The experiment could not be continued beyond this point because of the appearance of alarming symptoms of vasomotor collapse, abdominal and muscular cramps, generalized weakness and mental confusion. At this stage the concentration of urine chloride had fallen to 1.8 milliequivalents per liter, and the specific gravity pattern was definitely altered, all of the other determined substances contributing a substantially increased percentage of the total specific gravity. However, the subject was still able to produce a urine of high specific gravity, 1.0296, although chloride made up only 0.2 per cent of the total. The excretion of undetermined solids was not affected and the percentage of specific gravity accounted for remained at the control level. Since the diminution in salt excretion in these experiments was accompanied by a decrease in water excretion, while the outputs of other substances remained the same, the concentrations of all of these increased sufficiently to raise the specific gravity to a normal level, *i.e.*, the concentrating power of the kidney is unaffected and the hypochloremia in itself does not give rise to hyposthenuria.

Additional evidence for this was obtained from a patient with pyloric obstruction but normal kidney function (Subject C.) whose serum chlorides

had fallen to 84.0 milliequivalents per liter. In contrast to Subject Z., who showed marked clinical symptoms when serum chloride had fallen only to 96 milliequivalents per liter, there were no symptoms or signs associated with the severe hypochloremia other than a slight degree of dehydration. When fluids were withheld on a 33-hour concentration test, the final 12-hour urine specimen attained a specific gravity of 1.0245, although practically free of chloride. A 24-hour urine contained only 0.02 gram of chloride as sodium chloride, a concentration of 0.5 milliequivalent per liter, representing a chloride contribution to specific gravity of 0.1 per cent. Urea accounted for 34.1 per cent, more than would be expected from the 24-hour urea nitrogen excretion of 11.7 grams. Sulfate and phosphate contributions were also higher. Here again, the chloride deficit resulted in an increase in the specific gravity contribution of the other constituents.

Undetermined solid output in the 24 hours was 18.5 grams, the highest value thus far encountered. Organic acids were excreted in large amounts also, almost double the average normal output, but the significance of these high values is not apparent.

Another 24-hour urine was collected after the serum chloride level had been restored to normal (104 milliequivalents per liter). In this specimen chloride contributed a normal percentage of the specific gravity and the contributions of the other constituents were modified so that the usual pattern resulted (Table II). The output of organic acids and total solids dropped to normal levels so that the total percentage of specific gravity accounted for was somewhat greater than in the first specimen.

When both protein and salt intake are greatly restricted, the specific gravity pattern shows another change. Subject G. M. was an obese young woman on a reducing diet of extremely low caloric value and salt content. Although this diet placed the subject in a state of moderate ketosis, the urine containing appreciable quantities of acetone bodies, there was no evidence of acidosis either clinically or as shown by blood studies. Kidney function was normal and urine specific gravities above 1.025 were obtained on several occasions. On the day of urine collection the dietary intake consisted of 25 grams of protein, 11 grams of fat, 100 grams of carbohydrate and 0.7 gram of sodium chloride

with fluids restricted. Chloride contributed only 3.7 per cent of the specific gravity, sulfate 6.6 per cent, and urea 25.7 per cent. The urea contribution is higher than the protein intake would lead one to expect, again explicable on the basis of low salt excretion with accompanying reduction in water output. In this urine, one-half of the specific gravity is not accounted for, the greatest amount yet encountered. This value is consistent with the fraction of total solids undetermined, also one-half. The output of undetermined solids, in keeping with the low dietary intake, is low, 7.4 grams, despite a high concentration of organic acids, probably ketone bodies.

In this subject, a reduction in the amount of substances to be excreted did not result in hyposthenuria, as suggested by Alving and Van Slyke (6). Water excretion can be correspondingly reduced and a fairly concentrated urine can still be formed. In this case the 24-hour output of total solids was 15.1 grams, as compared to the normal range of 50 to 75 grams, but water output was only 250 cc., so that the resulting specific gravity was 1.0200.

In order to determine the variation in specific gravity pattern due to differences in the choice of diet of normal individuals, 24-hour urines from ten normal adults were studied. These subjects were all in good health, were ambulatory during the collection period, carried on their usual activities, and were allowed unrestricted choice of diet and fluids. The results are arranged in order of nitrogen output in Table III.

In these urines, the nitrogen output ranged from 7.1 to 17.1 grams and chloride output from 90 to 240 milliequivalents (5.3 to 14.0 grams as sodium chloride). The similarity of the specific gravity patterns, despite the variations in diet, is quite striking. It will be noted, also, that the total percentage of specific gravity accounted for, as well as the individual specific gravity contributions of the determined constituents, falls within the range found with the standard 40 to 50 gram and 100 to 110 gram protein diets used in the previous study. The correlation between protein intake and urea specific gravity contribution is not as clear-cut as in the previous experiments due to the greater variation in salt excretion; the latter, as shown above, bearing an inverse relationship to the contributions of the other constituents.

TABLE III

*Specific gravity pattern of urine of ten normal subjects with no dietary restrictions*

Subject	Sex	Date	Nitrogen output	Urine volume	Specific gravity	pH	Per cent of specific gravity due to:								Output undetermined solids
							Urea	Creatinine	Chloride	SO <sub>4</sub>	H <sub>2</sub> PO <sub>4</sub>	HPO <sub>4</sub>	HCO <sub>3</sub>	Total	
			grams	cc.	20°/14°										grams
J	F	October 25, 1939	7.1	1730	1.0079	5.79	15.7	1.7	31.9	8.0	7.7	0.9	0.3	66.2	15.4
D	F	November 28, 1939	7.8	550	1.0248	5.16	24.7	1.9	32.7	8.0	7.5	0.3		75.0	10.6
MB	M	November 20, 1939	8.1	602	1.0260	5.70	23.2	1.9	32.0	5.1	15.2	1.5	0.1	79.0	9.2
F	F	December 13, 1939	8.1	1015	1.0146	5.26	22.0	1.8	21.1	11.4	14.2	0.6	0.1	71.2	12.3
H	M	November 2, 1939	9.6	860	1.0265	5.62	18.1	1.8	36.3	11.0	8.9	0.8	0.1	77.0	13.3
IB	F	November 30, 1939	9.7	2060	1.0088	6.94	20.4	1.4	35.3	7.0	3.6	6.9	6.3	80.8	11.0
P	M	October 23, 1939	10.4	1645	1.0110	5.57	23.8	2.2	31.0	10.4	10.3	0.7	0.1	78.4	11.8
MP	F	December 15, 1939	10.7	1530	1.0127	5.78	24.0	0.9	34.3	11.6	9.2	1.1	0.1	81.2	9.8
R	M	December 18, 1939	12.3	2400	1.0095	6.61	21.5	1.3	24.9	10.5	8.9	8.1	1.4	76.6	14.6
W	M	December 4, 1939	17.1	1530	1.0191	6.08	25.4	1.5	30.0	10.4	9.9	2.4	0.4	80.0	16.8

The excretion of undetermined solids in these urines is also within the range found with the standard diets, except for one of 16.8 grams just above the upper limit previously found. There is no correlation between the output of undetermined solids and body weight or surface area.

In order to determine the ability of the kidney to produce marked variations in urine composition and specific gravity pattern under conditions of stress, the following experimental procedure was adopted:

Two subjects with normal kidney function were maintained at rest in bed on a varied diet but restricted to 50 grams of protein and 5 grams of added salt daily. For each experiment, the subject was placed on a standard diet with the same protein and salt content which was not varied for 3 days. Fluid as desired was permitted on the first day but was limited to 600 cc. on the second day, and to 200 cc. at breakfast on the third day. The 12-hour urine from 7 p.m. of the third day to 7 a.m. of the fourth day was collected (Specimen I). An intravenous injection of a hypertonic solution of urea or a salt was then given over a period of 10 minutes and, beginning 20 minutes later, one (Z.) or two (S.) 3-hour urine specimens (II and III) were collected. The substances used were 25.0 grams of urea, an approximately osmotically equivalent amount of sodium chloride (12.2 grams) or one-half this osmotic equivalent of sodium sulfate (9.85 grams) or sodium phosphate (8.45 grams of Na<sub>2</sub>HPO<sub>4</sub> and 2.05 grams of NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O, pH = 7.4), in each case dissolved in 50 cc. of water. As a control, 50 cc. of 5 per cent glucose solution were injected in one experiment with each subject. No food or fluid was allowed until the close of the urine collection periods.

Blood specimens were taken at the close of Period I with both subjects, at the beginning and end of period II with Subject Z., and at the midpoints of Periods II and III with Subject S. Cell volume was determined on whole blood and the sera were analyzed for urea, crea-

tinine, total protein, chloride, sulfate, and phosphate. Only the injected substance showed a marked change in concentration in the serum. Slight changes in the other constituents could be attributed to a small increase in water content.

Table IV summarizes the pertinent data from these experiments, giving, in addition to the specific gravity contributions of the determined substances, the urine flow, the specific gravity, the percentage of total solids determined and the blood level of the experimental substance at the end of Period I and at the midpoints of Periods II and III.

Period I, in all experiments, represents urine collected under similar conditions and shows a fairly constant specific gravity pattern consistent with the previously presented data for 24-hour urines. The total percentage of specific gravity determined is also fairly constant, ranging from 72 per cent to 79 per cent with Z., and from 69 per cent to 72 per cent with S. The urine output also varies only between narrow limits and is in the lower range of normal rates of excretion, so that a satisfactory concentrating ability is shown in all of these specimens.

Period II shows the changes resulting from the injection of a hypertonic solution when the body is already dehydrated. With both subjects, all four substances cause a significant increase in urine output. Despite the fact that urea and chloride were given in approximately iso-osmolar quantities, while only one-half this osmolar equivalent of sulfate and phosphate was injected, the order of diuretic effect is sulfate > phosphate > urea > chloride. This difference is not evident



TABLE IV  
Effect of intravenous injection of hypertonic solutions on specific gravity pattern of urine

Injected substance.....	Control			Urea			Chloride			Sulfate			Phosphate		
Period.....	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
SUBJECT Z.															
Volume, cc. per hour.....	25.5	33.2		18.5	50.7		17.2	50.7		29.2	120.7		23.1	93.2	
Specific gravity, 20°/4°.....	1.0240	1.0256		1.0261	1.0161		1.0282	1.0240		1.0231	1.0306		1.0260	1.0228	
Per cent specific gravity due to:															
Urea.....	20.4	17.5		18.3	44.0		15.2	12.5		17.8	7.9		19.2	10.0	
Creatinine.....	2.1	1.9		3.1	1.7		2.9	1.3		2.0	0.6		2.4	0.8	
BCl.....	33.9	36.0		27.1	27.9		25.2	44.5		36.1	10.2		32.3	6.1	
B <sub>2</sub> SO <sub>4</sub> .....	9.1	6.5		10.3	4.6		10.6	3.8		7.5	65.0		9.7	2.5	
BH <sub>2</sub> PO <sub>4</sub> .....	10.1	2.1		12.5	3.2		12.1	0.9		8.3	0.5		14.0	23.2	
B <sub>2</sub> HPO <sub>4</sub> .....	3.0	10.4		2.5	2.5		5.1	10.1		5.7	3.8		1.0	45.9	
BHCO <sub>3</sub> .....	0.4	4.6			1.2		0.6	14.8		1.2	6.7			3.3	
Per cent specific gravity determined.....	79.0	79.0		73.8	85.1		71.7	87.9		78.6	94.8		78.6	91.8	
Per cent total solid determined.....		75.1		68.7	82.8		64.6	82.5		74.6	89.6		72.1	83.6	
				Urea N (mgm. per 100 cc.)			Chloride (mEq. per liter)			Sulfate (mEq. per liter)			Phosphate (mEq. per liter)		
Serum concentration.....				17.1	26.8		104.2	110.6		0.96	5.12		2.82	8.55	
SUBJECT S.															
Volume, cc. per hour.....	26.6	18.9	33.9	11.4	64.8	71.7	19.8	54.1	45.9	14.1	98.4	39.7	16.5	96.4	50.3
Specific gravity, 20°/4°.....	1.0231	1.0228	1.0251	1.0258	1.0158	1.0181	1.0256	1.0224	1.0236	1.0262	1.0313	1.0345	1.0271	1.0211	1.0264
Per cent specific gravity due to:															
Urea.....	19.0	17.9	17.4	25.3	56.0	52.8	24.3	19.3	17.3	20.2	8.5	9.1	23.7	8.9	9.9
Creatinine.....	1.9	2.1	1.9	2.7	1.5	1.4	2.1	1.4	1.4	1.9	0.7	1.1	2.2	0.7	1.2
BCl.....	35.9	44.1	40.5	19.2	14.7	19.6	26.5	49.5	52.1	27.0	5.0	6.8	18.4	4.1	3.8
B <sub>2</sub> SO <sub>4</sub> .....	10.6	9.0	8.8	12.7	5.2	5.0	11.7	6.2	5.9	13.9	76.8	64.6	13.5	3.0	4.6
BH <sub>2</sub> PO <sub>4</sub> .....	8.7	8.2	12.5	13.3	3.8	4.0	12.2	3.3	5.5	12.9	3.2	6.0	17.4	58.8	35.1
B <sub>2</sub> HPO <sub>4</sub> .....	1.1	0.2	0.4	0.9	1.0	2.8	0.7	4.0	3.0	1.3	0.2	0.6	1.3	15.7	29.8
BHCO <sub>3</sub> .....					0.2	0.8		1.6	1.3						0.9
Per cent specific gravity determined.....	77.2	81.5	81.5	74.1	82.2	86.4	77.5	85.3	86.5	77.3	94.4	88.2	76.6	91.2	85.3
Per cent total solid determined.....	72.4	75.1	78.8	69.1	82.4	82.8	72.2	82.3	80.1	69.5	86.2	79.6	70.4	86.0	78.2
				Urea N (mgm. per 100 cc.)			Chloride (mEq. per liter)			Sulfate (mEq. per liter)			Phosphate (mEq. per liter)		
Serum concentration.....				14.4	37.4	32.2	101.5	108.1	106.3	0.78	3.96	2.20	2.64	6.56	3.31

from the specific gravities which show the most marked lowering with urea and even an increase with sulfate. In the two subjects, the diuresis produced by urea results in a specific gravity decrease of 0.0100, that by phosphate of 0.0032 and 0.0060, that by chloride of 0.0033 and 0.0032, while the diuresis due to sulfate is accompanied by specific gravity increases of 0.0072 and 0.0051. Thus the effect of salt or urea diuresis on specific

gravity depends on the nature of the solute as well as on the increased volume.

With the increase in blood level of the injected substance, it would be expected that the urine would show an increase in concentration of this substance with a decrease in concentration of the other constituents because of the diuresis. This is found to be the case, as is shown by the changes in specific gravity pattern. The one exception is

bicarbonate whose concentration in the second urine is uniformly increased due to an increase in pH. This morning "alkaline tide" effect has been shown to vary appreciably among individuals and to be independent of food intake (7). It is more evident in Subject Z. than in Subject S. The net result of all of these changes is a great distortion of the specific gravity pattern and a concomitant increase in the percentage of specific gravity determined. In the case of sulfate and phosphate, 91 per cent to 95 per cent of the observed specific gravity has been accounted for.

Period III, representing the second 3-hour period after the injection, shows qualitatively the same picture as Period II but some quantitative differences. In the two periods the specific gravity pattern remains about the same in the urea and chloride experiments while, with sulfate and phosphate, the pattern begins to show a return to the control level. The reason for this is made clear from the excretion data (not given in the table). In round figures, one-fourth of the injected urea is excreted in the first 3-hour period and an equal amount in the second. Similarly, one-sixth of the chloride introduced is eliminated in each of the periods. On the other hand, about 80 per cent of the sulfate is excreted in 3 hours and practically all of the rest in the next 3 hours, while the figures for phosphate are 55 per cent and 25 per cent. The decreased output of the two latter substances in the second 3-hour period is reflected in the decrease in the percentage of specific gravity determined.

Sufficient data are not available to justify a lengthy discussion of the output of undetermined solids in the short periods involved in these experiments. The overnight excretion (Period I) was quite constant, 4.3 to 4.9 grams with Subject Z. and 4.1 to 4.8 grams with Subject S. During Period II, the excretion ranged from 1.4 to 1.9 grams, equivalent to a somewhat greater output when compared to Period I on the same time basis. During Period III, the output (S.) was 1.2 to 1.6 grams. Under normal conditions, the output of undetermined solids is always greater during the day than during the night, which has been interpreted as due to a greater concentration of exogenous metabolites of digestion in the day specimen. Since these subjects, however, had no food until the end of the urine collections, it is

possible that diuresis due to salts or urea is responsible for a sweeping out of undetermined substances over a short time interval, which would not be apparent in a collection extending over a longer period. Also, since the calculation of undetermined solids is subject to considerable error, involving as it does all of the errors of the urine analysis, small variations over a short period may be exaggerated or masked. The nature of the undetermined solid is apparently not affected. The specific gravity factor per gram of undetermined solid per liter ranges from 0.00026 to 0.00043, with an average of 0.00035 (as compared with an average of 0.00039 previously found), with the variations apparently entirely random and showing no consistent upward or downward trend in passing from Period I to Period III.

TABLE V

*Effect of intravenous injection of urea and salts on their contributions to specific gravity*

	Normal range per cent	After injection per cent
Urea.....	15-25	44-56
Chloride.....	18-36	45-52
Sulfate.....	8-14	65-77
Phosphate.....	10-19	65-75

Table V shows clearly the marked change in the specific gravity contributions which were obtained in these experiments. It is quite possible that these are approaching maximal values under the experimental conditions because, in one subject, the injection of 75 grams of urea in three doses raised the contribution of urea to 58 per cent, only slightly higher than resulted when 25 grams were given, although the blood level reached 64 milligrams of urea nitrogen per 100 cc.

It must be emphasized that despite optimal conditions for achieving a high specific gravity in the urine, *i.e.*, low urine flow and high blood concentration, the highest specific gravity obtained in these experiments was 1.0345. There exists some very effective factor that limits the concentration of the urine. Smith (8) raises the question of two limiting factors to the concentration "ceiling", an "osmotic ceiling" (urea, sucrose, etc.) and an "ionic strength ceiling" (inorganic salts), the maximal concentration representing a resultant of these two effects. This question, as well as the

problem of water excretion raised by Gamble (9) requires further investigation.

The ability of the normal kidney, however, to vary the composition and specific gravity pattern of urine below the concentration "ceiling" is clearly shown. Under normal conditions of dietary intake and in health, the kidney eliminates a urine of remarkably uniform composition but it also has a large reserve capacity to take care of changes in the internal environment. How the damaged kidney performs under similar conditions will be dealt with subsequently.

#### CONCLUSIONS

1. The normal kidney under the usual conditions of diet produces a urine of remarkably constant composition and specific gravity pattern.

2. Large variations in the intake of water, fat, and carbohydrate have little effect on the specific gravity pattern.

3. On the other hand, marked changes in protein and salt intake may cause large variations in the composition of urine with a resultant distortion of the specific gravity pattern.

4. When large doses of salts or urea are given intravenously, the injected substance appears in high concentration in the urine and may account for at least 50 per cent to 75 per cent of the specific gravity.

5. The undetermined solids of urine, which account for the undetermined specific gravity, increase with the protein intake but may also be affected by other factors.

6. Chloride depletion *per se*, produced either experimentally or by pyloric obstruction, does not result in hyposthenuria.

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# DISTRIBUTION OF ASCORBIC ACID BETWEEN CELLS AND SERUM OF HUMAN BLOOD<sup>1</sup>

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In a previous report (1) the transfer of ascorbic acid from serum to blood cells *in vitro* was studied with concentrations of ascorbic acid in serum significantly higher than those observed *in vivo*. This communication deals with the distribution of ascorbic acid between the two phases of human blood when the vitamin is added in amounts above and within physiological limits. The distribution of ascorbic acid under conditions in which the concentrations in cells far exceed those in serum, and the partition of ascorbic acid added to blood under these conditions also are presented.

## METHODS

Ascorbic acid in serum was determined according to the recently described modification (1) of Mindlin and Butler's method (2). Whole blood was analyzed as before, according to the method of Emmerie and van Eekelen (3).

Recently, Butler and Cushman described a new method

by which the ascorbic acid concentration of whole blood filtrates is determined colorimetrically (4). Their conclusions confirm our observations (5) that the stores of vitamin C in the body can be estimated better by analyses of whole blood than by analyses of plasma or serum. At the same time, these authors object to the method used in this laboratory because the color developed in filtrates prepared according to the directions of Emmerie and van Eekelen fades rapidly. We agree with Mindlin and Butler (2) and Butler and Cushman (4) that accurate colorimetric determination of ascorbic acid is possible only if conditions can be established in which a stable color is developed.

Figures 1 and 2 of Butler and Cushman (4) are intended to show that in filtrates obtained by Emmerie and van Eekelen's procedure the color fades progressively while it is more stable in filtrates prepared according to their own directions. In these figures, when extrapolated to zero time, 1.16, 0.1 and minus 0.02 mgm. per cent by the Butler and Cushman method would correspond to 1.26, 0.18 and 0.18 mgm. per cent by the Emmerie and van Eekelen method. Extrapolation of an irregularly progressing curve is highly unsuitable and values thereby

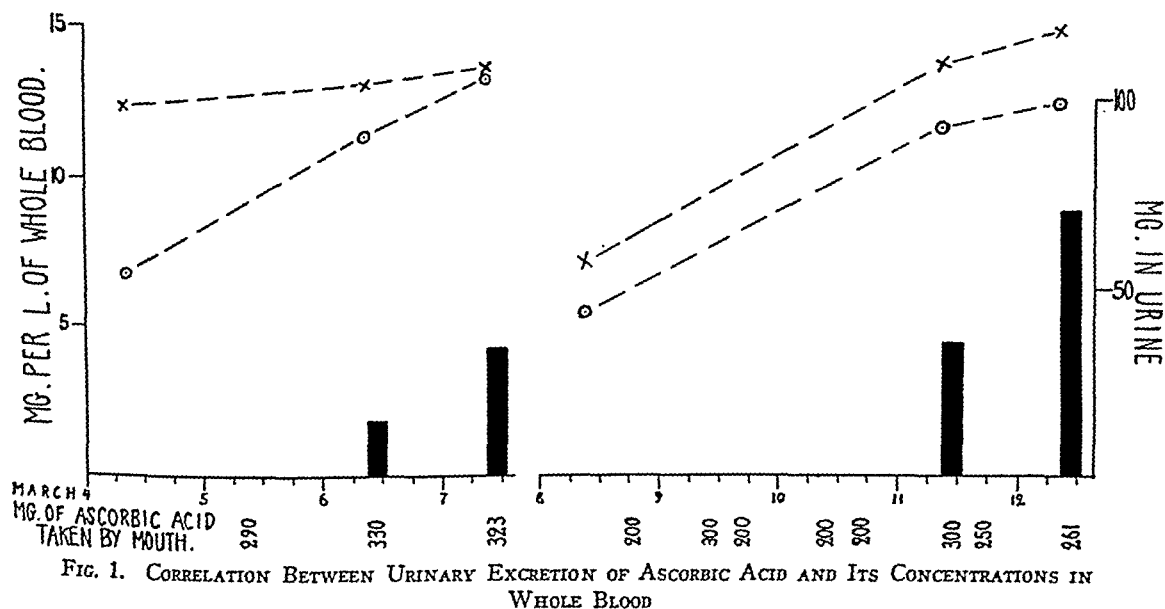


FIG. 1. CORRELATION BETWEEN URINARY EXCRETION OF ASCORBIC ACID AND ITS CONCENTRATIONS IN WHOLE BLOOD

Analyses of whole blood by the method of Emmerie and van Eekelen, X—X, by the method of Butler and Cushman, O—O.

On March 5, 6, 7, 9, 11 and 12 urinary surplus excretion was determined for 4 hours following the test doses. The amounts excreted are indicated by the solid columns. There was no surplus excretion on March 5 and 9.

<sup>1</sup> Aided by a grant from the Markle Foundation.

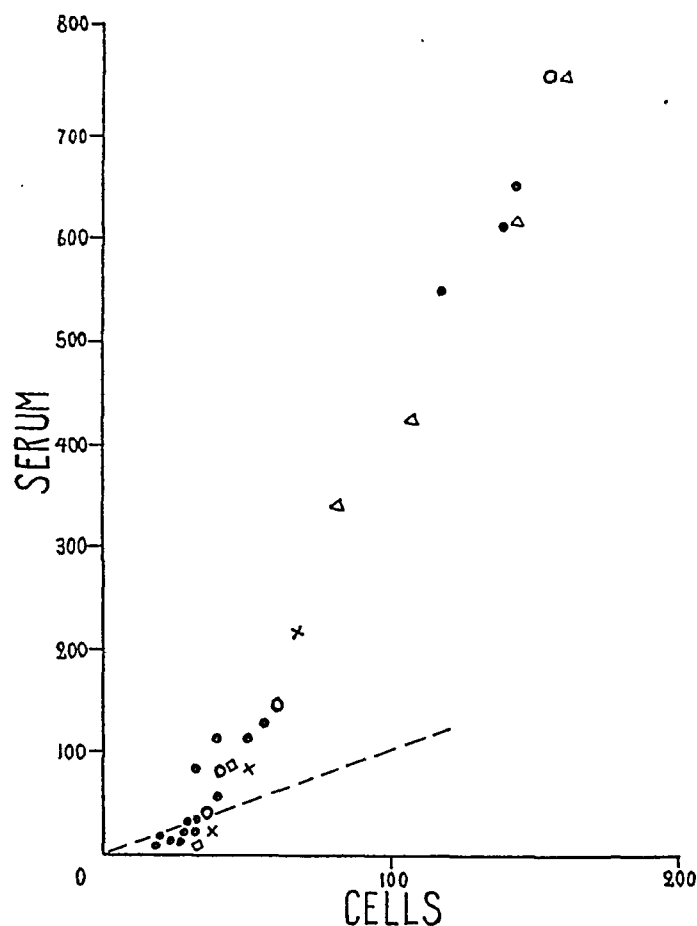


FIG. 2. DISTRIBUTION OF ASCORBIC ACID BETWEEN CELLS AND SERUM AT DIFFERENT CONCENTRATIONS OF ASCORBIC ACID

Concentrations in serum and cells are given as mgm. per liter of water. The water content was not determined but calculated on the basis of average figures of 72.3 per cent for cells and 93.5 per cent for serum, derived from data presented by Eisenman, Mackenzie and Peters (10). The dotted line represents the locus of points on which concentrations in cells and serum would be equal.

obtained are most dubious. Yet, if rapidly fading colors are to be evaluated at all, extrapolation is inevitable.

Figure 1 presents the relationship observed in two experimental subjects of about equal size between urinary excretion of ascorbic acid and whole blood concentrations determined according to both methods.<sup>2</sup> Emmerie and van Eekelen's procedure was used in its original form, *i.e.*, with titration. Filtrates prepared according to Butler and Cushman's method (4) were measured only with sodium 2, 6-dichlorobenzenoneindophenol as indicator; the methylene blue modification was not employed. The results secured by both methods agree fairly well with each other. Only in one instance—on March 4th, experiment 1—was a difference noted when Butler and Cushman's method gave 6.9 mgm. per liter, a value sig-

nificantly lower than 12.5 mgm. per liter obtained with Emmerie and van Eekelen's method.

The latter appeared to indicate better the degree of saturation, since after only 290 mgm. of ascorbic acid had been taken on the next day, 330 mgm. on March 6th caused surplus urinary excretion. With an initial concentration of only 6.9 mgm. per liter in whole blood, it is to be expected that a much larger dose of ascorbic acid would be required to produce saturation. On March 7th and March 11th (Figure 1) when Emmerie and van Eekelen's procedure gave identical results, approximately equal amounts of ascorbic acid were excreted in the urine; the correlation between blood concentrations and amounts excreted in urine is less satisfactory when the values obtained by Butler and Cushman's procedure are accepted.

In the analyses presented in Figure 1, the filtrates prepared according to the directions of Butler and Cushman did not develop stable colors. Expressed in terms of mgm. per liter, the rates of fading were from 0.8 to 1.1, 1.6 to 2.6, 1.8 to 2.5, 1.1 to 1.8, 1.6 to 2.4, 1.6 to 2.3 at 15 to 60 seconds. Plotted on a similar scale, the resulting curves would resemble those obtained by Butler and Cushman in Emmerie and van Eekelen's procedure and represented in their Figure 2. According to Butler and Cushman's criteria, the shape of these curves suggests the presence of interfering colorigenic materials. Our observations differ from their own data and interpretations only quantitatively, proving that their method is open to the same objections voiced against Emmerie and van Eekelen's procedure. This continued fading cannot be attributed to the development of color at an unsuitable acidity. The strength of metaphosphoric acid solutions was well adjusted by titration with 0.1 *N* sodium hydroxide. In blanks, stable colors invariably developed with solutions in which the acid was 0.21 to 0.22 *N* by titration, using phenolphthalein as indicator. Filtrates from whole blood had consistently the same normality (0.21) when the deproteinization had been carried out with 2 cc. of 2.0 *N* solution of metaphosphoric acid, 2 cc. of blood and 12 cc. of distilled water. The only modification of Butler and Cushman's directions consisted in the use of defibrinated instead of oxalated blood. The former was found to contain approximately 25 per cent less granulocytes than the latter. This difference in white cell count may be significant in certain instances. Observations by Stephens and Hawley (6), Cuttle (7), and Kreuzwendedich von dem Borne (8)—the latter using Emmerie and van Eekelen's method—that granulocytes contained high concentrations of ascorbic acid, were fully confirmed by Butler and Cushman's investigations (4). Since in the present study all measurements by both methods have been conducted on samples of blood treated identically, this loss of granulocytes in the process of defibrination was of no importance.

Two series of data derived incidentally from experiments not intended for this purpose further substantiate the accuracy of Emmerie and van Eekelen's method. Considering the number of manipulations and calculations

<sup>2</sup> Through the courtesy of Miss M. Cushman and Dr. A. M. Butler, the writer had the privilege of seeing and studying their method in advance of its publication.

involved, theoretical and observed values agree closely. In the first experiment, the original concentration in serum was 11.7 and that in cells 13.5 mgm. of ascorbic acid per liter, respectively; immediately following the addition of ascorbic acid, the serum concentration had increased to 18.6 mgm. per liter; after 45 minutes of incubation under the conditions described, this concentration had decreased to 15.5 mgm. per liter. With a cell volume of 46 per cent, 3.1 mgm. (18.6 minus 15.5) removed from serum and transferred to cells would correspond to 6.7 mgm. per liter of cells which, plus the original content of 13.5, would make a total cell concentration of 20.2 mgm. per liter. The calculated whole blood concentration was 17.7 (460 cc. of cells = 9.3 mgm. plus 540 cc. of serum = 8.4 mgm.). By analysis a concentration of 16.8 mgm. per liter of whole blood was found. In the second observation, the original concentration in serum was 15.2, that in cells, 18.3 mgm. per liter, respectively; after reducing the ascorbic acid content of serum by a procedure described below, a concentration of 4.0 mgm. per liter was found. With a cell volume of 48 per cent, whole blood should contain 10.88 mgm. per liter (8.78 mgm. in 480 cc. of cells plus 2.1 mgm. in 520 cc. of serum). By analysis 11.1 mgm. per liter were found.

#### EXPERIMENTAL RESULTS

Collection of blood, control of temperature, addition of crystalline ascorbic acid, continuous agitation of blood in atmospheres of nitrogen or oxygen were all carried out strictly according to the directions recently described (1). "Separated" and "true" sera also were obtained and are referred to in the same manner as before (1). Again, hemolysis and change in cell volume were completely prevented unless, as in one group of experiments, they are expressly mentioned. Investigating "the preservation of ascorbic acid in drawn samples of blood," Kassan and Roe (9) confirmed the necessity of avoiding hemolysis and the comparative stability of ascorbic acid in whole blood. These authors also state that ordinary glass containers promote hemolysis and consequently are not suitable for the preservation of ascorbic acid in blood. In view of this observation it is emphasized that pyrex glass was used throughout all our experiments.

It was previously demonstrated that an atmosphere of nitrogen stabilizes ascorbic acid in whole blood. To confirm the protective action of nitrogen, ascorbic acid was determined at certain intervals not only in true but also in separated sera. That ascorbic acid remains stable in separated sera is conclusively proved by previous experi-

ments (Figure 1, *B* and *C* (1)). After many similar observations had attested to the stability of ascorbic acid under these conditions, it seemed justifiable to discontinue determinations of ascorbic acid in "separated sera" in the further course of this investigation.

The technique of studying transfers of ascorbic acid in the experiments presented in this paper was the same throughout: continuous agitation at 37° C. (or at 7° C.) in an atmosphere of nitrogen. Crystalline ascorbic acid was dissolved in a small volume of serum and kept at 37° C. under nitrogen before addition. The amounts transferred to cells were expressed as mgm. per liter of cells and calculated from  $\frac{S_1 - S_2}{CV}$ , where  $S_1$  is the concentration in true serum immediately after addition,  $S_2$  the concentration in true serum after agitation, and  $CV$  the cell volume.

As shown before, this method of calculating cell concentrations proved to be reliable when checked by calculating cell concentrations from determinations in whole blood and serum and measurements of cell volumes. The latter procedure was used also for evaluating concentrations originally present in the cells of samples of blood used for further experimentation.

After the addition of ascorbic acid to whole blood its concentration was determined in samples of true serum removed immediately, after 30 minutes, and after about 45 minutes. In agreement with the previous report, transfers were invariably completed within 30 minutes. The results obtained after these intervals, therefore, should not and actually did not differ. Whenever they do, either a leakage in the system through which nitrogen is conducted or imperfectly cleaned glassware must be suspected. For the evaluation of observations resulting from this experimental technique, the prevention of oxidative destruction of ascorbic acid by nitrogen acquires intrinsic significance. Anticipating experimental data presented below, the protective action of nitrogen was evident in all the experiments in which no changes in ascorbic acid concentrations occurred in true sera separated from incubated whole blood before and after the aforementioned intervals. Furthermore, when ascorbic acid was added to samples of the same blood, but with different cell volumes (obtained by removing varying volumes of serum), the decrease in true serum varied directly with the number of cells. If ascorbic acid

were destroyed under the experimental conditions, the smallest amounts would be lost in the presence of the largest number of cells, the protective action of which upon ascorbic acid in serum is generally recognized.

*A. Rate of distribution of ascorbic acid between serum and cells following its addition to defibrinated blood<sup>3</sup>*

Figure 2 represents the distribution of ascorbic acid between the two phases of blood following the addition of widely varying amounts of the vitamin. In this figure, squares, triangles, open circles and crosses depict four experiments in which different amounts of ascorbic acid were added to samples of the same blood; solid circles indicate single observations. The data of both groups demonstrate alike that the amounts of ascorbic acid transferred to cells decrease as the concentrations in serum diminish.

*B. Lack of transfer of ascorbic acid from cells to serum*

A patient with peptic ulcer and vitamin C depletion subsequent to dietary treatment was found to have concentrations of ascorbic acid in whole blood of 4.6, in serum of only 0.61 mgm. per liter. At a cell volume of 41 per cent, the concentration of ascorbic acid in cells amounted to 10.3 mgm. per liter. These concentrations did not change after 2 hours of incubation under the usual conditions. The concentration in true serum remained constant also during 50 minutes following the addition of small amounts of ascorbic acid by which the concentration in serum was increased to 7.2 mgm. per liter.

Conditions similar to those of this observation were duplicated experimentally in the following way: From freshly drawn defibrinated blood, serum and cells were separated by centrifuging. The cells were kept in a tightly stoppered container at about 23° C. while the serum was treated with a wet stream of oxygen at 37° C. for 1½ to 2 hours, which almost completely destroyed its ascorbic acid. Consequently, serum and cells were placed separately in an atmosphere of nitrogen at 37° C. for 30 minutes before being

remixed. In the following presentation of experiments where this technique was employed, serum treated with oxygen, and subsequently with nitrogen, will be referred to as "prepared serum;" separated cells, kept at room temperature and treated with nitrogen for half an hour, as "prepared cells."

It will be noted that concentrations in serum of blood composed of "prepared serum" and "prepared cells" were low, but not zero. This was due to the impossibility of completely removing the original serum containing ascorbic acid from "prepared cells." This amount of ascorbic acid in the serum of "prepared cells" became even more inconvenient in the experiments in which it was increased by addition of ascorbic acid. As will be shown, such sera could be diluted to satisfactorily low concentrations by using 20 cc. each of "prepared serum" (without ascorbic acid) and "prepared cells."

In three such experiments, concentrations of ascorbic acid in true serum determined immediately and at intervals after mixing both phases of blood remained unchanged during 1 hour. The corresponding concentrations in serum and cells were 3.2 and 26.4, 4.3 and 20.0, 1.0 and 19.6 mgm. of ascorbic acid per liter of water, respectively.

In three more experiments, "prepared serum" was obtained from one portion of blood. Another portion was incubated following addition of ascorbic acid and the amounts transferred to cells were calculated in the manner described above. These amounts plus the ones originally present express the total concentration in cells. After removing the serum from this incubated whole blood as completely as possible by centrifugation, the remaining cells were treated with nitrogen for 30 minutes. In this way, "prepared cells" containing 37.1, 144.3 and 117.4 mgm. of ascorbic acid per liter of water, respectively, were remixed with corresponding "prepared sera" containing 11.8, 35.7 and 36.0 mgm. per liter of water, respectively. In no instance was any change in the concentrations in true sera observed during 70 minutes of continuous agitation at 37° C. under nitrogen. In three experiments carried out identically, except that the temperature was 7° C., concentrations in true sera likewise remained stable.

<sup>3</sup> Human blood was used invariably.

*C. Lack of transfer of ascorbic acid from serum to cells following the addition of this substance*

Incidentally, the distribution of added ascorbic acid was investigated in the blood of a patient with secondary anemia who was markedly depleted as the result of inadequate diet. The original concentrations were 4.4 mgm. per liter of whole blood, 1.0 mgm. per liter of serum; cell volume was 31 per cent, the concentration of ascorbic acid per liter of cells 11.97 mgm., or 16.6 mgm. per liter of water in cells, against 1.07 mgm. per liter of water in serum. In experiments carried out in duplicate no uptake of ascorbic acid could be detected during 1 hour following the addition of ascorbic acid so as to increase the serum concentrations to 57.0 and 61.0 mgm. per liter of water, respectively.

This incidental observation was imitated by using blood composed of "prepared serum" and "prepared cells." Ascorbic acid was added to samples of this blood (a) immediately, (b) 1 hour and (c) 2 hours after its two components had been re-mixed. Determining the amounts transferred to the cells in the course of 1 hour, the corresponding changes in serum concentrations were (a) 73.3 to 65.3, (b) 59.0 to 54.4 and (c) 68.9 to 68.0 mgm. per liter, respectively. In another experiment, one part of serum was transformed into "prepared serum" while the other part, kept under nitrogen, maintained its original concentration of 8.5 mgm. of ascorbic acid per liter. Consequently, "prepared cells" from the same blood were added to these sera and gently agitated under the usual conditions. After 2 hours ascorbic acid was added to each blood. In the blood with serum, the ascorbic acid of which had been preserved, a transfer of 8.3 (63.3 to 55.0) mgm. = 20.2 mgm. per liter of cells was noted after 60 and also after 105 minutes; cells in contact with "prepared serum" for 2 hours prior to the addition of ascorbic acid took up no ascorbic acid; the concentration in the serum remained 48.3 mgm. per liter of serum throughout. These results must be presented with certain reserve since cell volumes did not remain constant, changing in the experiments from 40 to 41.5 per cent, 40 to 41.8 per cent, and 40 to 41 per cent, respectively. The increases in cell volume, significant because

they never occurred in the experiments hitherto described, appear to be due to changes in serum rather than in cells. They occurred in "prepared serum" as well as in serum treated with nitrogen only, and were independent of the addition of ascorbic acid; they could not be prevented by using cells separated from samples of blood drawn freshly from the same subject after the preparation of the sera was completed.

#### DISCUSSION

The data presented in Figure 2 show that the amounts of ascorbic acid transferred from serum to cells, following its addition to blood, bear a direct relation to its concentrations in serum. The ratios of distribution between cells and serum are about the same in experiments in which different concentrations of ascorbic acid are added to samples of the same blood as they are when ascorbic acid is added to various bloods. At concentrations of ascorbic acid higher than any that occur *in vivo*, serum always contains more than cells. The ratio, ascorbic acid of serum: ascorbic acid of cells, declines from approximately 4.75 at serum concentrations of 600 to 750 mgm. per liter to 2.5 at concentrations of 100 to 150 mgm. per liter and to 1.5 at a concentration of 50 mgm. per liter. It approaches unity, as previous experiments suggested (1) at about 35 mgm. of ascorbic acid per liter of water in serum.

Previously, the necessity of imitating physiological conditions was demonstrated with respect to temperature and to the avoidance of sedimentation of blood (1). Yet, in spite of maintaining proper temperature and providing for contact between all the cells and serum by continued agitation, the distribution ratios differed from those observed *in vivo* and it may now be concluded that this was due to artificially high concentrations. When these also conformed to physiological limits (Figure 2, below dotted line), however, the ratios *in vitro* corresponded to those established *in vivo*.

Below 35 mgm. per liter of water the concentrations in cells exceed those in serum. That reversal of the ratio appears at about this point is confirmed by the experiment presented in Table I in which different quantities of ascorbic acid were added to samples of the same blood to yield vari-



TABLE I

*Distribution of different concentrations of ascorbic acid added to sample of the same blood*

	Serum	Cells	Quotient $\frac{\text{Serum}}{\text{Cells}}$
	<i>mgm. per liter of water</i>	<i>mgm. per liter of water</i>	
Original	11.8	19.0	
After addition	18.2	20.0	0.91
After addition	26.7	29.0	0.92
After addition	38.0	32.1	1.18

ous concentrations in the region of 35 mgm. per liter.

These observations *in vitro* agree well with results of analyses of serum and cells of freshly drawn blood reported earlier (5). In thirty instances without exception more ascorbic acid was found in cells than in serum. The differences, illustrated in Figure 3 of that communication, would be even greater if concentrations were recorded in terms of mgm. per liter of water instead of mgm. per liter of total volume. *In vitro* as well as *in vivo*, at concentrations within the physiological range, cells withdraw the vitamin from serum beyond the point of equal distribution. Above the critical concentration of 35 mgm. per liter, however, which is far higher than concentrations encountered in circulating blood, the amounts transferred are so small that the concentrations ultimately attained are always lower in cells than in serum. This inversion of distribution ratios with changing concentrations seems to prove quite conclusively that simple diffusion alone cannot control the partition of ascorbic acid between the two phases of blood.

In freshly drawn blood, concentrations of ascorbic acid never exceed 35, rarely 20 mgm. per liter, unless they are intentionally raised for experimental purposes. *In vivo*, as stated before, distribution ratios, expressed as concentration in serum: concentration in cells, are always less than 1.0. Ratios greater than 1.0 may be produced by the administration of large doses of ascorbic acid; but only transiently, because of the transfer to cells and the rapid elimination by the kidneys which sets in when the concentration in the serum surpasses about 14 mgm. per liter.

If a curve were drawn to represent the trend of all the observations in Figure 2, it would not be possible to determine with any certainty the

trend of distribution ratios at high concentrations. In the experiments recorded in this figure the maximum concentration attained in cells, when the concentration in serum had been raised to 750 mgm. per liter of water, was approximately 160 mgm. per liter of water. No further increase in concentrations in the cells could be detected when the concentrations in serum were increased to about 900 mgm. However, at these concentrations filtrates must be diluted about 100 times for analysis. The error of the method, therefore, prohibits exact estimations. For this reason it is impossible to decide whether 160 mgm. per liter of water actually represent the maximal load which cells will assume. The general trend of the observations does suggest, however, that the concentrations in cells approach a limit.

As concentrations diminish, the curve describing the distribution of points seems to approach the origin. This is substantiated by observations of Butler and Cushman (4), who reported minimal concentrations of ascorbic acid both in cells and serum in patients with scurvy or prolonged vitamin C deficiency. Saturation is characterized by high concentrations of ascorbic acid in both phases of blood (5); scurvy by its almost complete absence in serum as well as in cells (4).

Passage of ascorbic acid from cells to serum was not observed under conditions in which its concentration was definitely higher in cells than in serum. Transfers from serum to cells appeared to be associated with some metabolic activity of the cells, since they took place at 37° C., but not at 7° C. But variations of temperature or increasing concentrations in cells to values as high as 140 mgm. per liter of water had no power to move the vitamin in the other direction, from cells to serum. Ascorbic acid, therefore, seems to form a combination with some substance or substances within the cells from which it cannot be released by any measures thus far discovered. A similar conclusion has been reached by Reedman and McHenry (11), Fujita and Ebihara (12) and Holtz and Walter (13) concerning the state of the vitamin in plant and animal tissues. These authors assume that it is combined with protein. Levine, Marples and Gordon (14), from studies on infants have concluded that the metabolism of aromatic amino acids depends upon the quantities of ascorbic acid stored in the body.

The avidity of cells for ascorbic acid and their stubborn retention of the substance *in vitro* conform well to what is known of their behavior in the body. Individuals have tolerated deprivation of vitamin C for 42 (4), 94 (15) and 160 (16) days without the appearance of any signs of scurvy. Butler and Cushman (4) noted that the concentration of ascorbic acid in the serum diminished rapidly early in the course of vitamin C deprivation, while the concentrations in the cells decreased considerably later, and gradually. The granulocytes—and possibly platelets—retained ascorbic acid longer than red cells. If ascorbic acid cannot pass from cells to serum this would indicate that ascorbic acid is metabolized or destroyed more rapidly in erythrocytes than in granulocytes.

The principles that govern the distribution and transfer of ascorbic acid in blood provide an adequate explanation for the fact already established, that the amounts of ascorbic acid in the body can be better estimated from analyses of whole blood than from analyses of serum (4, 5). Concentrations in cells cannot be neglected. In Table IV of Butler and Cushman's (4) paper there are five instances in which, without scurvy, plasma was free from ascorbic acid. Yu (18) reported that with equally low vitamin C concentrations (less than 2 mgm. per liter of plasma) some persons had manifest scurvy while others were apparently healthy. Under similar circumstances, in our experience, an explanation for the discrepancy has been found by analyses of whole blood.

Passage of ascorbic acid from serum to cells was lacking in a blood with an abnormally low serum concentration. A similar observation was omitted from our previous report (1) because it was made at the very beginning of this investigation when the experimental technique was not yet perfected. Transfer of ascorbic acid did not take place either in blood, in the serum of which it had been destroyed experimentally. Cells were found to lose the ability to take up ascorbic acid not immediately after being placed in "prepared serum" but only progressively in the course of 2 hours. Except for this observation that the reaction is not an instantaneous one, the effect of low concentrations of ascorbic acid in the environment upon the membrane, metabolic activity or

physicochemical status of cells cannot even be intimated. It may be correlated with observations by van Eekelen (15) who found that the amounts of vitamin C daily metabolized diminished as the stores in the body decreased. Since concentrations diminish more rapidly in serum than in cells, and since low serum concentrations impair the ability of cells to take up ascorbic acid, it does not seem inconceivable that the metabolism of ascorbic acid in tissue cells like that in blood cells depends upon concentrations in serum.<sup>4</sup>

In the example just discussed, the influence of serum upon cell function was related to concentrations of ascorbic acid in the former; the effect of incubated serum upon cell volumes cannot be correlated with any known factor.

#### SUMMARY AND CONCLUSIONS

(1) Amounts of ascorbic acid transferred from serum to cells were estimated from concentrations in the former removed from whole blood immediately and at certain intervals after the addition of ascorbic acid.

(2) As concentrations in serum decrease, the amounts transferred to and the concentrations attained in cells also diminish.

(3) Distribution ratios are greater than unity above serum concentrations of about 35 mgm. per liter of water, smaller than unity below this concentration. The partition of ascorbic acid in both phases of blood is not governed by simple diffusion.

(4) Ascorbic acid is not transferred from cells to serum when the former contain concentrations significantly higher than the latter, either at 37° C. or at 7° C. Ascorbic acid appears to be irreversibly combined with some intracellular substance or substances.

(5) The magnitude of concentrations of ascorbic acid in serum seems to affect the cellular functions involved in the mechanism of transfer of added ascorbic acid. No uptake occurred when cells, prior to addition, were kept in serum with low concentrations of ascorbic acid.

<sup>4</sup> Differences in the rate of uptake by different tissues (19) or with regard to dehydroascorbic acid and its reduced form (20) would not contradict this inference.

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# THE RENAL EXCRETION OF INORGANIC PHOSPHATE IN RELATION TO THE ACTION OF VITAMIN D AND PARATHYROID HORMONE<sup>1</sup>

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The mode of action of vitamin D in the prevention and cure of rickets is still not understood. In 1921, Howland and Kramer (1) pointed out that the concentration of phosphorus in the serum was usually reduced in human rickets, at times to very low levels, although the concentration of calcium might be within normal limits. This has been confirmed repeatedly and it has been found that the administration of vitamin D results in a prompt rise in the concentration of phosphorus, even to abnormally high levels with excessive dosage. The mechanism through which vitamin D influences the concentration of phosphorus in the body fluids has not been satisfactorily explained. In balance experiments in man (2, 3) and experimental animals (4) it has been shown that during states of vitamin D deficiency absorption of calcium and phosphate from the intestinal tract is reduced and that the administration of vitamin D increases the absorption of these ions. In the rat, the studies of Nicolaysen (5) have indicated that in the absence of vitamin D the absorption of calcium is primarily diminished and that the absorption of phosphate is only secondarily affected. Careful examination of the data from metabolic studies in the rachitic infant leads to the conclusion that deficient absorption of phosphate from the intestinal tract cannot wholly explain the diminished concentration of phosphate in the plasma. In infants developing rickets on a cow's milk diet, *i.e.*, a diet high in both calcium and phosphorus, the amount of phosphate absorbed from the intestinal tract would be sufficient for the needs of the infant were the phosphate retained rather than excreted in the urine.

The excretion of phosphate in the urine must play an important rôle in the regulation of the concentration of phosphate in the body fluids.

How is the renal excretion of phosphate controlled and does vitamin D influence the concentration of phosphate in the blood plasma through an effect on the excretion of this ion by the kidneys? The present experiments were conducted to answer these questions.

## *The Renal Excretion of Phosphate*

Walker (6) and Walker and Hudson (7) have shown that the phosphate of the plasma is filterable through the glomerular membranes of the amphibian. These workers have also found that under certain conditions active reabsorption of phosphate takes place in the proximal tubules so that the urine in the distal tubules and bladder may be almost phosphate-free. There was no evidence that phosphate was secreted into the lumen of the tubules.

In the dog, Pitts (8) has shown that the clearance of phosphate increases as the concentration of phosphate in the plasma increases and approaches the xylose clearance at very high levels of plasma phosphate. At normal levels of phosphate in the blood plasma the urine may be almost phosphate-free and the excretion of phosphate in the urine is only a small fraction of that filtered through the glomeruli. In the mammalian kidney, therefore, reabsorption of phosphate must also take place as the glomerular filtrate passes through the renal tubules.

In order to study this tubular reabsorption of phosphate quantitatively, simultaneous determinations of the creatinine and phosphate clearances were made in the dog following the intravenous injection of phosphate salts.

## METHODS

Female dogs fed standardized diets were used for the experiments. The studies of phosphate excretion were made about 18 to 20 hours after the last feeding. The

<sup>1</sup> Read in abstract before the Society for Pediatric Research, May 1, 1940.

animals were unanesthetized and lay comfortably on the animal board with loose restraints. A solution of 0.1 molar sodium phosphate of approximately pH 7.4 was injected intravenously in a dosage of 7 to 10 cc. per kilogram of body weight, together with creatinine (0.3 gram per kgm.). In most of the experiments 300 cc. of water were given by stomach tube in order to increase the urine volume, thus making the collections more accurate. The animals were catheterized and urine was collected quantitatively during successive periods of 10 to 20 minutes each. The bladder was washed with measured volumes of distilled water at the end of each period. Repeated blood samples were taken from the external jugular vein during the periods of urine collection. All analyses were done on the separated serum and the same analytical methods were used for serum and urine. Creatinine was determined by the usual Jaffé reaction (9); inorganic phosphate, by the Fiske and Subbarow method (10). A photoelectric colorimeter was used.

In order to calculate the clearances of creatinine and phosphate, the concentrations as determined in the serum were plotted on semi-logarithmic paper and the concentrations at the mid-point of each period obtained from the curve. The plasma clearances were then calculated by the usual formula  $C = \frac{UV}{P}$ . It has been demonstrated that the creatinine clearance in the dog can be used as a measure of the rate of glomerular filtration (11). If the plasma phosphate is completely filtrable through the glomeruli, the differences between the clearances of creatinine and those of phosphate measure the rate of reabsorption of phosphate by the renal tubules.

Following the injection of phosphate salts it has been shown, however, that a colloidal phosphate complex is formed which is not filtrable through collodion membranes (12). This non-ultrafiltrable fraction of the phosphate rapidly disappears from the plasma if no further phosphate is given. Repeated studies of the filtrability of the serum phosphate through collodion membranes were made following the intravenous injection of sodium phosphate in the amounts described above. The membranes were prepared by the method of Greenberg and Gunther (13) and the apparatus used for ultrafiltration was that described by Benjamin and Hess (14). The results of such experiments are shown in Table I.

It may be seen that soon after the concentration of phosphate in the plasma is raised by the intravenous injection, an appreciable fraction of the inorganic phosphate is not filtrable through collodion. In about 60 to 90 minutes, however, the phosphate is again completely or almost completely filtrable. This is true even in the experiments in which the serum calcium was raised above the normal value by the administration of vitamin D or parathyroid extract. In the studies of renal function, the determinations of the clearances of phosphate were started 60 minutes fol-

TABLE I  
*Filtrability of plasma inorganic phosphate through collodion membranes following intravenous injection of sodium phosphate*

Experiment	Serum calcium	Time elapsed	Phosphate		Filtrable
			Plasma water	Ultrafiltrate	
	<i>mgm. per 100 cc.</i>	<i>minutes</i>	<i>mgm. per 100 cc.</i>		<i>per cent</i>
1	9.0	82	10.8	9.9	92
		163	9.9	9.8	99
2	10.5	21	9.2	8.1	88
		50	7.0	6.9	99
		95	6.1	5.9	97
		144	6.2	5.8	94
3*	11.9	63	8.4	8.0	95
		97	5.9	6.0	100
4*	14.6	63	8.6	7.2	84
		97	6.5	6.1	94
		136	5.9	6.0	100
		177	5.3	5.3	100
5†	14.5	31	10.6	8.0	76
		71	6.9	6.7	97
		111	6.5	6.1	95
6‡	16.0	63	7.4	6.5	88
		96	5.6	4.9	89
		134	5.0	5.1	100
		178	4.9	5.0	100

\* Following administration of vitamin D.

† Following administration of dihydrotachysterol (A. T. 10).

‡ Parathyroid extract 2 cc. injected preceding day.

lowing the intravenous injection of phosphate salts and continued for about 2 hours. After this interval the plasma phosphate is essentially completely filtrable through collodion membranes and it is assumed that it is also completely filtrable through the glomerular membranes.

The results of typical experiments are shown in Tables II, III and IV. The creatinine clearances are seen to remain relatively constant in the successive periods. The clearances of phosphate, however, decrease as the concentration of phosphate in the plasma decreases and in some cases the excretion of phosphate in the urine may be reduced almost to zero. If the creatinine clearance be taken as the rate of glomerular filtration, since the concentration of phosphate in the plasma is known, the amount of phosphate filtered through the glomeruli per minute may be calculated. The quantity of phosphate reabsorbed by the renal tubules is obtained by subtraction of the

TABLE II

The reabsorption of phosphate by the renal tubules  
(Dog B. Weight 9.6 kgm.)

Period	Total elapsed time	Urine volume	Creatinine clearance	Plasma phosphate	Phosphate clearance	Phosphate		
						Filtered	Excreted	Reabsorbed
	minutes	cc. per minute	cc. per minute	mgm. per 100 cc.	cc. per minute	mgm. per minute		
	0							
1	60-70	1.9	35.0	4.6	5.7	1.61	0.26	1.35
	72-80	1.25	35.0	4.6	5.7	1.61	0.26	1.35
2	120-140	1.8	38.5	9.4	23.2	3.62	2.18	1.44
3	140-160	0.55	31.0	7.3	15.5	2.26	1.13	1.13
4	160-170	0.8	36.0	6.6	17.0	2.38	1.12	1.26

TABLE III

The reabsorption of phosphate by the renal tubules  
(Dog A. Weight 7.7 kgm.)

Period	Total elapsed time	Urine volume	Creatinine clearance	Plasma phosphate	Phosphate clearance	Phosphate		
						Filtered	Excreted	Reabsorbed
	minutes	cc. per minute	cc. per minute	mgm. per 100 cc.	cc. per minute	mgm. per minute		
1	0-10							
	12-20		23.5	3.3	2.4	0.78	0.08	0.70
2	70-90	1.45	26.2	6.5	14.0	1.70	0.91	0.79
3	90-110	0.45	22.3	5.5	9.8	1.23	0.54	0.69
4	110-130	0.25	22.7	5.0	8.8	1.14	0.44	0.70
5	130-150	0.20	22.6	4.6	7.4	1.04	0.34	0.70
6	150-170	0.20	23.9	4.5	6.0	1.08	0.27	0.81
7	170-190	0.20	23.0	4.4	5.0	1.01	0.22	0.79

amount excreted in the urine from the amount filtered. If such calculations are made, it may be seen that the tubular reabsorption of phosphate expressed as milligrams of phosphorus per minute is essentially constant and is not influenced by the elevation of the concentration of phosphate in the serum. In many of the experiments the calculated tubular reabsorption of phosphate is found to vary to some extent with fluctuations in the creatinine clearance, suggesting that the reabsorption of phosphate is in part affected by changes in the filtration rate. The present experiments cannot answer this point since the tubular reabsorption of phosphate is determined indirectly. Any errors in the determination of the creatinine clearance would produce an error in the same direction in the calculation of the phosphate reabsorption.

If the tubular reabsorption of phosphate remains constant as the concentration of phosphate in the plasma decreases, a concentration should be reached at which the quantity filtered equals the quantity reabsorbed and no phosphate should be excreted in the urine. This estimated concentration of phosphate will be termed the equilibrium concentration and may be calculated by the following formula:  $C_E = \frac{T_m}{F} \times 100$ , where  $C_E$  is the equilibrium concentration,  $T_m$  is the maximal rate of reabsorption of phosphate and  $F$ , the rate of glomerular filtration. The reabsorption of phosphate by the renal tubules may not be complete and traces may be present in the urine at concentrations of phosphate in the plasma below the calculated equilibrium concentration. In the dog, at least, in those experiments in which the concentration of phosphate in the plasma decreased to the calculated equilibrium value, the excretion of phosphate in the urine dropped to less than 0.002 milligrams per minute, indicating that less than 0.1 per cent of the phosphate filtered escaped reabsorption.

The rate of reabsorption of phosphate is not affected by water diuresis. In these experiments

TABLE IV

The reabsorption of phosphate by the renal tubules  
(Dog C. Weight 9.9 kgm.)

Period	Total elapsed time	Urine volume	Creatinine clearance	Plasma phosphate	Phosphate clearance	Phosphate		
						Filtered	Excreted	Reabsorbed
	minutes	cc. per minute	cc. per minute	mgm. per 100 cc.	cc. per minute	mgm. per minute		

EXPERIMENT I

	0-10	75 cc. 0.1 M phosphate intravenously						
1	50-70	0.45	43.3	9.8	7.6	4.24	0.74	3.50
2	70-90	0.9	42.3	9.4	5.1	3.97	0.48	3.49
3	90-110	1.9	43.4	9.2	5.0	3.99	0.46	3.53
4	110-130	1.8	43.1	9.0	4.1	3.88	0.37	3.51
5	130-150	1.3	40.6	8.9	2.6	3.61	0.23	3.38

EXPERIMENT II

	0-10	75 cc. 0.1 M phosphate intravenously						
1	55-75	0.1	39.5	9.5	3.1	3.75	0.29	3.46
2	95-115	0.2	38.7	9.0	1.2	3.48	0.11	3.37
3	115-135	0.15	35.7	8.9	0.5	3.18	0.04	3.14
4	135-155	0.35	41.3	8.8	0	3.63	0	3.63

the rate of urine excretion was varied from 0.1 to 5 cc. per minute without significant changes in the tubular reabsorption of phosphate. This is in accord with previous reports that the urinary excretion of phosphate is not increased by water diuresis (15).

In experiments in the young dog extending over many months, variations in the renal clearances of phosphate are observed which are apparently related to age. The concentration of plasma inorganic phosphate in the young dog is much higher than that in the adult animal. This is similar to the findings reported in man. Anderson and Elvehjem (16) have also recently found that the concentration of plasma inorganic phosphate in the dog decreases with age. Comparative studies of the creatinine clearances and renal tubular reabsorption of phosphate in dogs from 2 to

TABLE V

*The influence of age upon the concentration of phosphate in the serum and the renal tubular reabsorption of phosphate*

Dog	Age	Weight	Creatinine clearance	Phosphate reabsorbed	Equilibrium concentration	Serum * phosphorus
	months	kgm.	cc. per minute	mgm. per minute	mgm. per 100 cc.	
A	4	5.7				9.6
	17	7.7	23.3	0.94	4.0	4.7
B	4					7.3
	13	9.0	31.1	1.43	4.6	4.9
	20	9.7	29.9	1.25	4.2	4.6
	27	9.7	32.9	1.04	3.2	3.4
C	3	7.7	29.1	2.30	7.9	7.7
	7	9.9	42.5	3.48	8.2	8.2
	8	10.6	43.9	3.46	7.9	7.7
	10	10.9	43.2	2.66	6.2	6.7
	12	10.7	38.2	1.79	4.7	5.9
D	2½	8.5	44.0	4.22	9.6	8.4

\* Blood taken 18 hours after last feeding.

27 months of age are shown in Table V. In the young animal the rate of tubular reabsorption of phosphate is greater in proportion to the rate of glomerular filtration than in the adult dog. In dog C during the latter part of the first year of life, at which time the filtration rate has reached a relatively constant level, there is a decrease in the rate of tubular reabsorption of phosphate from the maximum values, and the calculated equilibrium concentration of phosphate decreases

markedly. The data for dog B indicate that there is a gradual decrease in the rate of tubular reabsorption of phosphate during the second year of life, with no change in the filtration rate. In most of the experiments the concentrations of phosphate in the fasting state approximate the calculated equilibrium concentration.

### *The effect of vitamin D upon the renal excretion of phosphate*

A 6-week old female collie puppy was placed on a vitamin D free diet, low in both calcium and phosphorus. The composition of the diet was a modification of that described by Morgan (17). At the age of 20 weeks rachitic changes in the bones could be demonstrated by roentgenogram. The concentrations of calcium and phosphorus in the serum were both reduced. During the period of active rickets repeated studies of the tubular reabsorption of phosphate were carried out as described in the preceding section. The animal was then given 20,000 units vitamin D in the form of irradiated ergosterol each day for 3 days, totaling 60,000 units. On the fourth day, studies of the renal function were done and repeated at intervals of 1 to 2 weeks thereafter

TABLE VI

*The effect of vitamin D upon the renal tubular reabsorption of phosphate in the rachitic dog*

Date	Creat- inine clearance	Phos- phate re- absorbed	Equilib- rium concen- tration	Serum	
				Phos- phorus	Cal- cium
	<i>cc. per minute</i>	<i>mgm. per minute</i>	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	
1939					
October 2.....	Rachitogenic diet started—age 6 weeks				
October 26....				8.8	10.6
November 29..	29.1	2.30	7.9	7.7	12.7
December 22..	37.9	2.56	6.8	6.2	8.5
1940					
January 8....	43.1	2.40	5.6	5.7	8.4
January 22...	45.4	2.56	5.6	6.0	7.6
February 12..	20,000 units vitamin D				
February 13..	20,000 units vitamin D				
February 14..	20,000 units vitamin D				
February 15..	40.7	3.87	9.5	9.0	9.0
February 28..	43.1	3.47	8.1	8.2	10.5
March 27.....	44.1	3.46	7.9	7.4	9.6
April 25.....	45.0	3.19	7.1	7.2	8.5
June 3.....	50.2	3.12	6.2	6.6	8.6
June 16.....	80,000 units vitamin D				
June 17.....	49.9	3.49	7.0	6.6	9.3
June 28.....	45.3	2.91	6.4	6.7	10.2

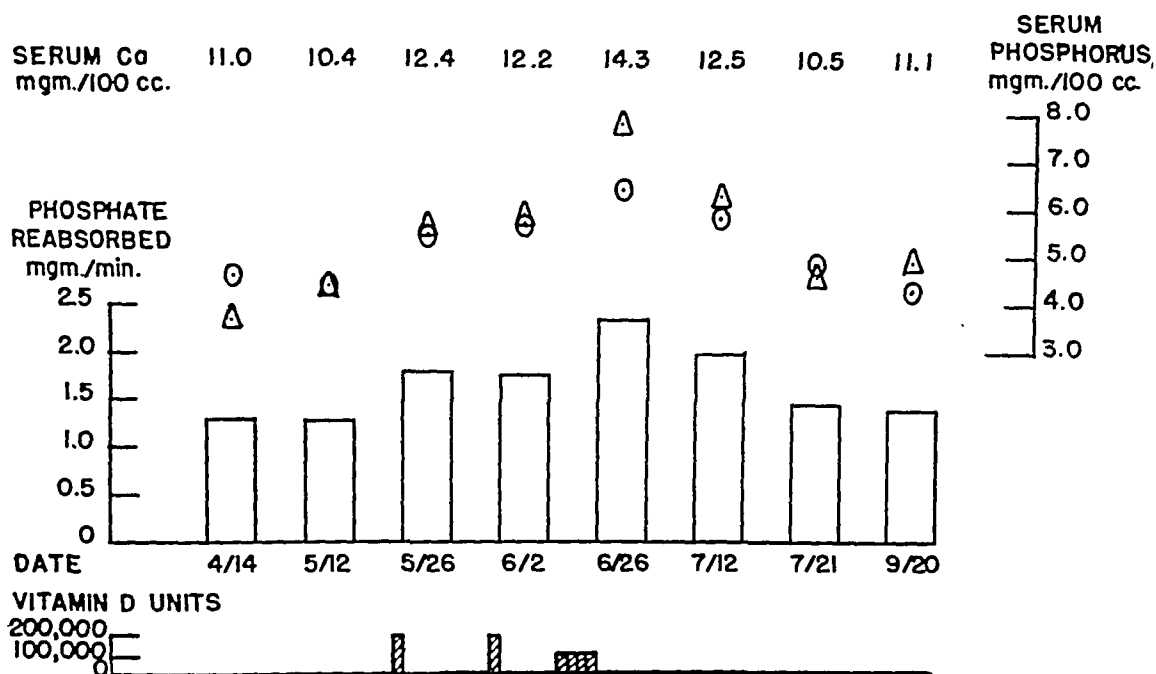


FIG. 1. THE EFFECT OF VITAMIN D ON TUBULAR REABSORPTION OF PHOSPHATE—MATURE DOG B

Columns represent the average tubular reabsorption of phosphate—expressed as mgm. of phosphorus per minute.

○ Concentration of serum phosphorus in fasting state.

Δ Calculated "equilibrium concentration" of phosphate expressed as mgm. phosphorus per 100 cc.

without any further treatment with vitamin D. The results are shown in Table VI. In this and succeeding tables, the data for each experiment represent the average of 5 or 6 successive periods of 20 minutes each.

It is evident that following the administration of vitamin D, there is a marked increase in the rate of reabsorption of phosphate by the renal tubules. The creatinine clearances remain unchanged. The calculated equilibrium concentration of phosphate is therefore increased from 6 mgm. per 100 cc. during the rachitic state to 9.5 mgm. per 100 cc. following treatment with vitamin D. The concentrations of phosphorus in the serum during the postabsorptive state parallel closely this calculated value. The data indicate that in these experiments the effect of vitamin D in raising the concentration of phosphorus in the serum is due to its action on the tubular reabsorption of phosphate, preventing loss of phosphate in the urine. The effect of the treatment with 60,000 units of vitamin D is seen to persist for several months with a gradual decrease in the rate of

tubular reabsorption of phosphate, until the pre-treatment level was reached. At this time further treatment with vitamin D was again effective in increasing the reabsorption of phosphate by the renal tubules, but to a much slighter extent than previously.

A second dog was studied in similar manner except that the animal was not started on the vitamin D free diet until 3 or 4 months of age and no evidences of rickets were demonstrated by roentgenogram. The animal had already become sexually mature before the renal function studies were done. Following the administration of vitamin D, however, an increase in the reabsorption of phosphate by the renal tubules was also found with an associated increase in the concentration of phosphate in the serum as shown in Figure 1. This effect could be demonstrated 24 hours after the animal had been given a single large dose of vitamin D (200,000 units). Following the administration of a total of 800,000 units of vitamin D, a still greater effect upon the renal tubular reabsorption of phosphate was found. With this



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TABLE V

*The influence of age upon the concentration of phosphate in the serum and the renal tubular reabsorption of phosphate*

Dog	Age	Weight	Creatinine clearance	Phosphate reabsorbed	Equilibrium concentration	Serum * phosphorus
	months	kgm.	cc. per minute	mgm. per minute	mgm. per 100 cc.	
A	4	5.7				9.6
	17	7.7	23.3	0.94	4.0	4.7
B	4					7.3
	13	9.0	31.1	1.43	4.6	4.9
	20	9.7	29.9	1.25	4.2	4.6
	27	9.7	32.9	1.04	3.2	3.4
C	3	7.7	29.1	2.30	7.9	7.7
	7	9.9	42.5	3.48	8.2	8.2
	8	10.6	43.9	3.46	7.9	7.7
	10	10.9	43.2	2.66	6.2	6.7
	12	10.7	38.2	1.79	4.7	5.9
D	2½	8.5	44.0	4.22	9.6	8.4

\* Blood taken 18 hours after last feeding.

27 months of age are shown in Table V. In the young animal the rate of tubular reabsorption of phosphate is greater in proportion to the rate of glomerular filtration than in the adult dog. In dog C during the latter part of the first year of life, at which time the filtration rate has reached a relatively constant level, there is a decrease in the rate of tubular reabsorption of phosphate from the maximum values, and the calculated equilibrium concentration of phosphate decreases

TABLE VI

*The effect of vitamin D upon the renal tubular reabsorption of phosphate in the rachitic dog*

Date	Creatinine clearance	Phosphate reabsorbed	Equilibrium concentration	Serum	
				Phosphorus	Calcium
	cc. per minute	mgm. per minute	mgm. per 100 cc.	mgm. per 100 cc.	
1939					
October 2.....	Rachitogenic diet started—age 6 weeks				
October 26.....				8.8	10.6
November 29..	29.1	2.30	7.9	7.7	12.7
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January 22....	45.4	2.56	5.6	6.0	7.6
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February 28..	43.1	3.47	8.1	8.2	10.5
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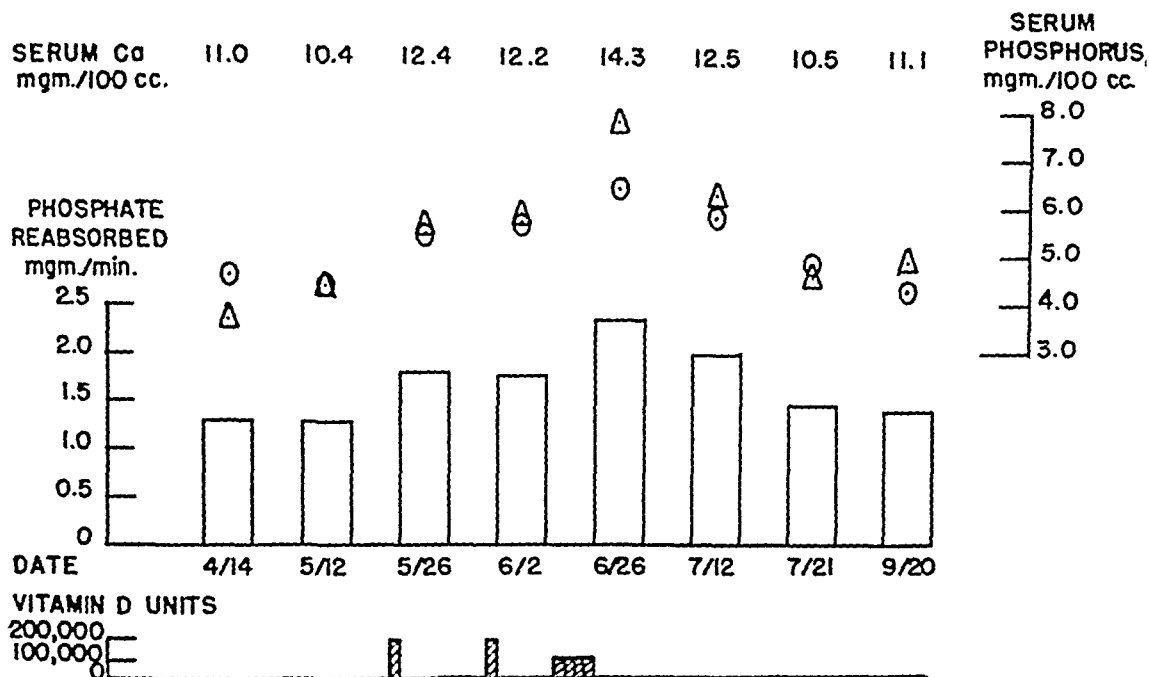


FIG. 1. THE EFFECT OF VITAMIN D ON TUBULAR REABSORPTION OF PHOSPHATE—MATURE DOG B

Columns represent the average tubular reabsorption of phosphate—expressed as mgm. of phosphorus per minute.

○ Concentration of serum phosphorus in fasting state.

Δ Calculated "equilibrium concentration" of phosphate expressed as mgm. phosphorus per 100 cc.

without any further treatment with vitamin D. The results are shown in Table VI. In this and succeeding tables, the data for each experiment represent the average of 5 or 6 successive periods of 20 minutes each.

It is evident that following the administration of vitamin D, there is a marked increase in the rate of reabsorption of phosphate by the renal tubules. The creatinine clearances remain unchanged. The calculated equilibrium concentration of phosphate is therefore increased from 6 mgm. per 100 cc. during the rachitic state to 9.5 mgm. per 100 cc. following treatment with vitamin D. The concentrations of phosphorus in the serum during the postabsorptive state parallel closely this calculated value. The data indicate that in these experiments the effect of vitamin D in raising the concentration of phosphorus in the serum is due to its action on the tubular reabsorption of phosphate, preventing loss of phosphate in the urine. The effect of the treatment with 60,000 units of vitamin D is seen to persist for several months with a gradual decrease in the rate of

tubular reabsorption of phosphate, until the pre-treatment level was reached. At this time further treatment with vitamin D was again effective in increasing the reabsorption of phosphate by the renal tubules, but to a much slighter extent than previously.

A second dog was studied in similar manner except that the animal was not started on the vitamin D free diet until 3 or 4 months of age and no evidences of rickets were demonstrated by roentgenogram. The animal had already become sexually mature before the renal function studies were done. Following the administration of vitamin D, however, an increase in the reabsorption of phosphate by the renal tubules was also found with an associated increase in the concentration of phosphate in the serum as shown in Figure 1. This effect could be demonstrated 24 hours after the animal had been given a single large dose of vitamin D (200,000 units). Following the administration of a total of 800,000 units of vitamin D, a still greater effect upon the renal tubular reabsorption of phosphate was found. With this

dosage a marked hypercalcemia developed. Following cessation of treatment, the renal tubular reabsorption of phosphate rapidly fell to the previous levels and the concentration of serum phosphorus in the fasting state likewise decreased. With the dosage of vitamin D used no effect upon the creatinine clearances was noted. However, in another dog given 1,600,000 units of vitamin D over a period of 8 weeks, the creatinine clearance decreased from approximately 25 cc. per minute to 15 cc. per minute. This effect upon glomerular filtration was not apparently associated with permanent injury to the kidney since the creatinine clearance again returned to normal levels following cessation of treatment. With the administration of excessive amounts of vitamin D to dogs, Goormaghtigh and Handovsky (18) have described anatomical changes in the kidney and hypertension. Although vitamin D is extremely effective in increasing the tubular reabsorption of phosphate in the growing dog depleted of vitamin D, only a slight effect may be produced upon the renal tubular reabsorption of phosphate in the normal adult animal, even though extremely large amounts, sufficient to raise the serum calcium to abnormally high levels, are given. In agreement with these observations are the reports that in the adult human as well as in the adult dog the concentration of phosphorus in the plasma is increased but slightly following the administration of vitamin D (19).

*The effect of parathyroid extract on renal tubular reabsorption of phosphate*

Many workers have attempted to correlate the physiological actions of vitamin D with those of the parathyroid hormone. Although both vitamin D and parathyroid extract produce an increase in the concentration of calcium in the serum, their effects upon the concentration of phosphate are diametrically opposite. Studies of the effects of parathyroid extract upon renal tubular reabsorption of phosphate are shown in Tables VII and VIII.

In each of the experimental animals a marked decrease in the renal tubular reabsorption of phosphate was found following the injection of parathyroid extract. No change in the creatinine clearance was noted with the amount of para-

TABLE VII

*Effect of parathyroid extract on renal tubular reabsorption of phosphate*

Treatment	Creat- inine clearance	Phos- phate re- absorbed	Equilib- rium concentration	Serum	
				Phos- phorus	Cal- cium
	<i>cc. per minute</i>	<i>mgm. per minute</i>	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	
Dog A					
Control . . . . .	23.5	0.89	3.8	4.1	11.0
Parathyroid extract 2 cc.*	25.2	0.57	2.3	3.0	14.1
Dog B					
Control . . . . .	30.9	1.37	4.4	4.6	10.5
Parathyroid extract 4 cc.*	30.9	0.89	2.9	4.0	14.7

\* Given subcutaneously in divided doses the day preceding the experiment.

TABLE VIII

*The effect of parathyroid extract on the renal tubular reabsorption of phosphate in rachitic dog*

Date	Treatment	Creatinine clearance	Phosphate reabsorbed	Equilibrium concentration	Serum	
					Phosphorus	Calcium
		cc. per minute	mgm. per minute	mgm. per 100 cc.	mgm. per 100 cc.	
January 22	Control	45.4	2.56	5.6	6.0	7.6
January 30	Parathyroid extract 6 cc.*	43.3	2.00	4.6	5.3	9.1
February 8	Control	42.5	2.55	6.0	6.2	8.4
February 15	Vitamin D 60,000 units	40.7	3.87	9.5	9.0	9.0

\* Given in divided doses during day preceding experiment.

thyroid extract used in these experiments. The calculated equilibrium concentrations of phosphate were markedly decreased from the control values. This effect of parathyroid extract could also be demonstrated in the rachitic dog, although relatively large amounts of extract were necessary to affect the renal excretion of phosphate (Table VIII). It may be seen that the already low rate of renal tubular reabsorption of phosphate in the rachitic dog is decreased still further with an associated decrease in the concentration of serum phosphorus. In contrast to the effect of para-

thyroid extract is shown the result of treatment with vitamin D, as previously described. The observed decrease in the rate of reabsorption of phosphate by the renal tubules following the injection of parathyroid extract explains the repeated observation of the increased urinary excretion of phosphate and diminished concentration of phosphate in the serum produced by treatment with parathyroid extract and in states of hyperparathyroidism. Conversely, the very high concentrations of phosphorus in the serum in hypoparathyroidism are presumably the result of increased tubular reabsorption of phosphate. When excessive amounts of parathyroid extract are injected, a marked decrease in the creatinine clearance may be observed with a return to normal following cessation of treatment. This effect of toxic doses of parathyroid extract in diminishing glomerular filtration may explain the secondary rise in serum phosphorus when excessive doses of parathyroid extract are injected. With a considerable decrease in glomerular filtration and the liberation of large amounts of phosphate from bone, the serum phosphorus may rise even though tubular reabsorption of phosphate is reduced.

#### DISCUSSION

"The kidneys appear to serve as the ultimate guardians of the constitution of the internal environment, which they maintain with increasing accuracy under most unfavorable circumstances." Peters (20) has thus emphasized the activities of the kidneys in preventing loss of essential solutes from the body fluids as well as in the elimination of substances present in excess. These observations apply to the function of the kidney in maintaining the concentrations of inorganic phosphate in the plasma and other body fluids. The quantity of phosphate filtered through the glomeruli per day is greatly in excess of the phosphate intake. However, the reabsorption of most of the phosphate from the glomerular filtrate as it passes through the renal tubules prevents the loss of excessive quantities of phosphate in the urine and thus allows for the maintenance of the normal concentrations of phosphate in the body fluids.

The experiments reported here demonstrate that under given conditions there is a maximal rate of tubular reabsorption of phosphate. When the

concentration of phosphate in the plasma is increased by the administration of phosphate salts, the quantity of phosphate filtered in excess of the reabsorptive capacity of the tubules is excreted in the urine. Shannon and Fisher (21) first demonstrated this phenomenon of a limiting maximal rate of tubular reabsorption in connection with the reabsorption of glucose and other sugars. Shannon (22) has postulated the theory that an intermediate compound is formed in the renal tubule cells which again dissociates, liberating the free solute into the body fluids, and that the rate of this reaction is the limiting factor in the reabsorption of certain solutes by the tubule cells.

The importance of the renal excretion of phosphate in regulating the concentration of this ion in the body fluids is shown clearly by the experiments reported here. The concentration of phosphate may, of course, be influenced by many factors other than the renal excretion, *e.g.*, the availability of phosphate for absorption from the gastro-intestinal tract, the movement of phosphate from the extracellular fluids into the cells or vice versa, the precipitation of calcium phosphate in the skeleton, or the mobilization of phosphate from the bones into the body fluids. At equilibrium, however, the concentration of phosphate in the plasma approaches the concentration at which the rate of reabsorption of phosphate by the renal tubules is approximately equal to the rate at which phosphate is filtered through the glomeruli.

It has been shown that following the administration of vitamin D there is a rapid increase in the tubular reabsorption of phosphate, the rate of glomerular filtration remaining unchanged. The increased concentration of phosphate in the body fluids produced by the administration of vitamin D can be explained as the result of this effect upon renal tubular function. The work of other investigators has shown that vitamin D influences the absorption of calcium, and secondarily of phosphate, from the intestinal tract. This action of vitamin D in conjunction with its effects upon renal function would result in sustained high concentrations of both calcium and phosphate in the body fluids, a condition favorable for rapid calcification. It is probable that the antirachitic potency of vitamin D is dependent on this com-

bined action on renal tubular function and intestinal absorption.

In this connection, cases of rickets which do not respond to vitamin D therapy are of considerable interest. One group of such cases has been reported (23) in which the concentration of phosphate in the serum is exceedingly low and is not increased by the administration of vitamin D. Studies of these patients reveal marked loss of phosphate in the urine, despite low concentrations of this ion in the blood plasma. These patients may also exhibit other evidences of renal dysfunction, such as renal glycosuria. Albright, *et al* (24) have reported a case of low phosphorus rickets refractory to treatment with vitamin D and associated with diffuse calcification of the kidneys. It is possible that the development of low phosphorus rickets in these cases is due to a failure of the renal tubular mechanisms concerned with the reabsorption of phosphate and that this tubular deficiency is not corrected by the administration of vitamin D. It is of further interest that many of these patients exhibit a severe chronic acidosis. Preliminary studies in the dog have shown that following the production of acidosis, the renal tubular reabsorption of phosphate is greatly diminished (25).

The demonstrated effect of parathyroid extract in diminishing the reabsorption of phosphate by the renal tubules is in agreement with earlier observations that parathyroid extract produces an increase in the excretion of phosphate in the urine, with a simultaneous decrease in the concentration of phosphate in the plasma. This effect may also be related to the changes found in rickets. Hyperplasia of the parathyroids has been observed post-mortem in cases of severe rickets (26), and Hamilton and Schwartz (27) have found evidence of an increased amount of parathyroid hormone in the blood of rachitic rabbits. Albright and Sulikowitch (28) have suggested that the low serum phosphorus found in rickets may be due to the secondary hyperparathyroidism present. The hyperplasia of the parathyroids in rickets has been explained as a compensatory response to the calcium deficiency which results from the failure of the intestinal tract to absorb the calcium. Recently Ham *et al* (29) have found that enlargement of the parathyroids could be produced in rats

by feeding diets low in calcium and deficient in vitamin D. However, when given a high calcium vitamin D free diet, the rats did not show enlargement of the parathyroids although they did develop low phosphorus rickets. In the experiments reported here the prompt increase in the tubular reabsorption of phosphate observed following the administration of vitamin D suggests that vitamin D exerts a direct effect upon the renal tubule cells.

#### SUMMARY

By means of the concurrent determinations of creatinine and phosphate clearances in the dog, following the intravenous injection of phosphate salts, it is possible to study quantitatively the reabsorption of phosphate by the renal tubules. It is found that under standard conditions there is a limiting maximal rate of reabsorption of phosphate by the renal tubules which does not vary when the concentration of phosphate in the plasma is elevated by the administration of phosphate salts. The phosphate filtered through the glomeruli which is in excess of the maximum which can be reabsorbed by the renal tubules is excreted in the urine.

The administration of vitamin D to young dogs who have been fed a rachitogenic diet produces a marked increase in the maximal rate of reabsorption of phosphate by the renal tubules, thus increasing the concentration of inorganic phosphate in the plasma at equilibrium. This effect is demonstrable 24 hours after adequate amounts of vitamin D are given and is probably an important factor in its antirachitic activity.

The effect of parathyroid extract upon the tubular reabsorption of phosphate is opposite to that of vitamin D. Following injections of parathyroid extract there is a considerable decrease in the rate of reabsorption of phosphate by the renal tubules and a consequent reduction in the concentration of phosphate in the plasma.

We are indebted to Mead Johnson and Co. for the irradiated ergosterol, and to Eli Lilly and Co. for the parathyroid extract used in these experiments.

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# STUDIES ON NEOPLASMS WITH THE AID OF RADIOACTIVE PHOSPHORUS. II. THE PHOSPHORUS METABOLISM OF THE NUCLEOPROTEIN, PHOSPHOLIPID AND ACID SOLUBLE FRACTIONS OF NORMAL AND LEUKEMIC MICE

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Previous studies (1, 2) have shown that the phosphorus metabolism of mouse tissues is markedly altered by leukemic infiltration, the absorption and retention of radio-phosphorus being increased in the tissues of leukemic animals. The present work goes one step further in elucidating the seat of this altered phosphorus metabolism by fractionating the tissues into the three broad groups of biologically active phosphorus compounds: the phospholipid, nucleoprotein, and acid soluble fractions.

## EXPERIMENTAL

Twenty-five male and twenty-five female mice of the highly inbred and genetically uniform Strong A strain and of the same age (seven weeks) and weight (16.5 to 17 grams) were inoculated intraperitoneally with 0.1 cc. of a suspension containing fifteen million leukemic cells (3). (This method of inoculation has the advantage of producing a localized tumor mass in the peritoneum and a very uniform generalized leukemic infiltration of the liver, spleen, and lymph nodes.) This lymphoma, which is highly vascular, non-necrotic, and has very little stroma, has been transferred about eighty times in the A strain of mice. It takes in 100 per cent of the mice inoculated, and regressions have not been observed. A similar group of twenty-five male and twenty-five female animals was retained as controls. Five groups of animals, consisting of five males and five females, respectively, with five corresponding groups of controls, were placed in twenty wire-bottomed cages.

The leukemic animals were fed Purina dog chow *ad lib*. The weighed daily food consumption of each group of leukemic animals determined the amount fed to its respective group of control animals on the following day. In addition, each group of five animals received six grams of whole oats per day and ten grams of fresh lettuce every other day. Thus the phosphorus and caloric intake of the leukemic and control animals was approximately the same.

The radio-phosphorus was administered by intraperitoneal injection of 0.5 cc. of a solution containing 7.5 mgm. sodium phosphate at pH 7.4 and emitting 5.5 micro-

curies<sup>1</sup> of beta activity at the time the first group was injected. Ten leukemic and ten normal animals were given this solution seven, five, three and one-half, two, and one-quarter days before all the animals were killed, which occurred on the eighteenth day after inoculation with leukemic cells. At this time the weights of leukemic and control animals were approximately the same.

At autopsy, all leukemic animals had peritoneal tumors weighing about 800 to 1,000 mgm. and had uniformly enlarged spleens, livers and lymph nodes—both central and peripheral. These tissues (tumor, liver, spleen and lymph nodes) from the animals of each group were pooled, weighed and prepared for analysis. The weighed tissue was ground thoroughly under 95 per cent alcohol with the aid of a little sand and transferred to 100 cc. centrifuge tubes. The carcasses of each normal and leukemic group were pooled, ashed and assayed for radioactivity.

## METHODS OF TISSUE FRACTIONATION

*A. Phospholipid.* The tissue phospholipid was removed by four successive extractions, the first with 95 per cent alcohol and the other three with 3:1 alcohol: ether. Seventy cc. of solvent were used in each extraction which was carried out at 50 to 55° C. under a reflux condenser. The tubes containing the tissue were centrifuged after each extraction and the solvent decanted. The total duration of extraction was thirty-six hours.

The combined alcohol and ether extracts were evaporated at low temperature to a volume of about 2 cc. Five cc. of water were added and the mixture transferred to a separatory funnel where it was extracted five times with 70 cc. portions of petroleum ether. The combined petroleum ether extracts were evaporated down to a small volume, transferred to ashing capsules, ignited, and set aside for analysis. The aqueous phase which contained only a small amount of activity was added to the acid soluble fraction, since it contained some of the simpler non-lipid compounds removed from the tissue in the course of the alcohol extractions.

<sup>1</sup> Preliminary experiments had shown that this amount of radioactivity administered to mice caused no observable metabolic changes which could be termed "radiation effects." The effects of increased doses of radioactive phosphorus upon the activity curves obtained in this type of study will be reported later.



TABLE I

*Differential uptake of labeled-phosphorus in various fractions of normal and leukemic mice*

Tissue	Normal or leukemic mice	Nucleoprotein			Phospholipid		Acid soluble		
		Per cent of dose* per gram	Per cent of normal	Per cent per mgm. P	Per cent of dose* per gram	Per cent of normal	Per cent of dose* per gram	Per cent of normal	Per cent per mgm. P
One-quarter day									
Tumor.....		0.92		0.32	0.14		1.51		1.96
Liver.....	L	0.96	192	0.43	0.83	37	1.36	105	1.70
Liver.....	N	0.50		0.30	2.18		1.30		1.83
Spleen.....	L	1.45	79	0.49	0.20	42	2.92	135	3.50
Spleen.....	N	1.83		0.68	0.48		2.17		3.02
Lymph nodes.....	L	1.22	188	0.43	0.23	92	2.42	197	3.31
Lymph nodes.....	N	0.65		0.27	0.25		1.23		1.89
Two days									
Tumor.....		3.08		1.07	0.60		0.92		1.19
Liver.....	L	1.97	346	0.90	0.96	86	0.95	122	1.19
Liver.....	N	0.57		0.34	1.11		0.78		1.10
Spleen.....	L	3.56	238	1.21	0.47	76	1.42	143	1.71
Spleen.....	N	1.49		0.56	0.62		0.99		1.38
Lymph nodes.....	L	3.39	346	1.19	0.47	109	1.10	183	1.51
Lymph nodes.....	N	0.98		0.40	0.43		0.60		0.92
Three and one-half days									
Tumor.....		2.68		0.93	0.47		0.84		1.09
Liver.....	L	1.60	262	0.73	0.75	89	0.83	160	1.04
Liver.....	N	0.61		0.36	0.84		0.52		0.73
Spleen.....	L	2.70	212	0.92	0.47	85	0.88	116	1.06
Spleen.....	N	1.27		0.47	0.55		0.76		1.05
Lymph nodes.....	L	2.70	375	0.95	0.49	148	0.75	150	1.02
Lymph nodes.....	N	0.72		0.30	0.33		0.50		0.77
Five days									
Tumor.....		2.22		0.77	0.40		0.67		0.87
Liver.....	L	1.43	251	0.66	0.63	126	0.64	160	0.80
Liver.....	N	0.57		0.34	0.50		0.40		0.56
Spleen.....	L	2.31	240	0.78	0.47	152	0.81	150	0.98
Spleen.....	N	0.96		0.34	0.31		0.54		0.75
Lymph nodes.....	L	2.36	303	0.83	0.33	97	0.79	376	1.08
Lymph nodes.....	N	0.78		0.32	0.34		0.21		0.32
Seven days									
Tumor.....		1.85		0.64	0.35		0.60		0.78
Liver.....	L	1.06	258	0.49	0.53	147	0.54	186	0.67
Liver.....	N	0.41		0.25	0.36		0.29		0.41
Spleen.....	L	1.82	243	0.62	0.31	155	0.63	140	0.76
Spleen.....	N	0.75		0.28	0.20		0.45		0.62
Lymph nodes.....	L	1.61	256	0.57	0.33	143	0.84	400	1.15
Lymph nodes.....	N	0.63		0.27	0.23		0.21		0.32

\* These figures represent the amount of activity found in the fractions obtained from one gram fresh tissue.

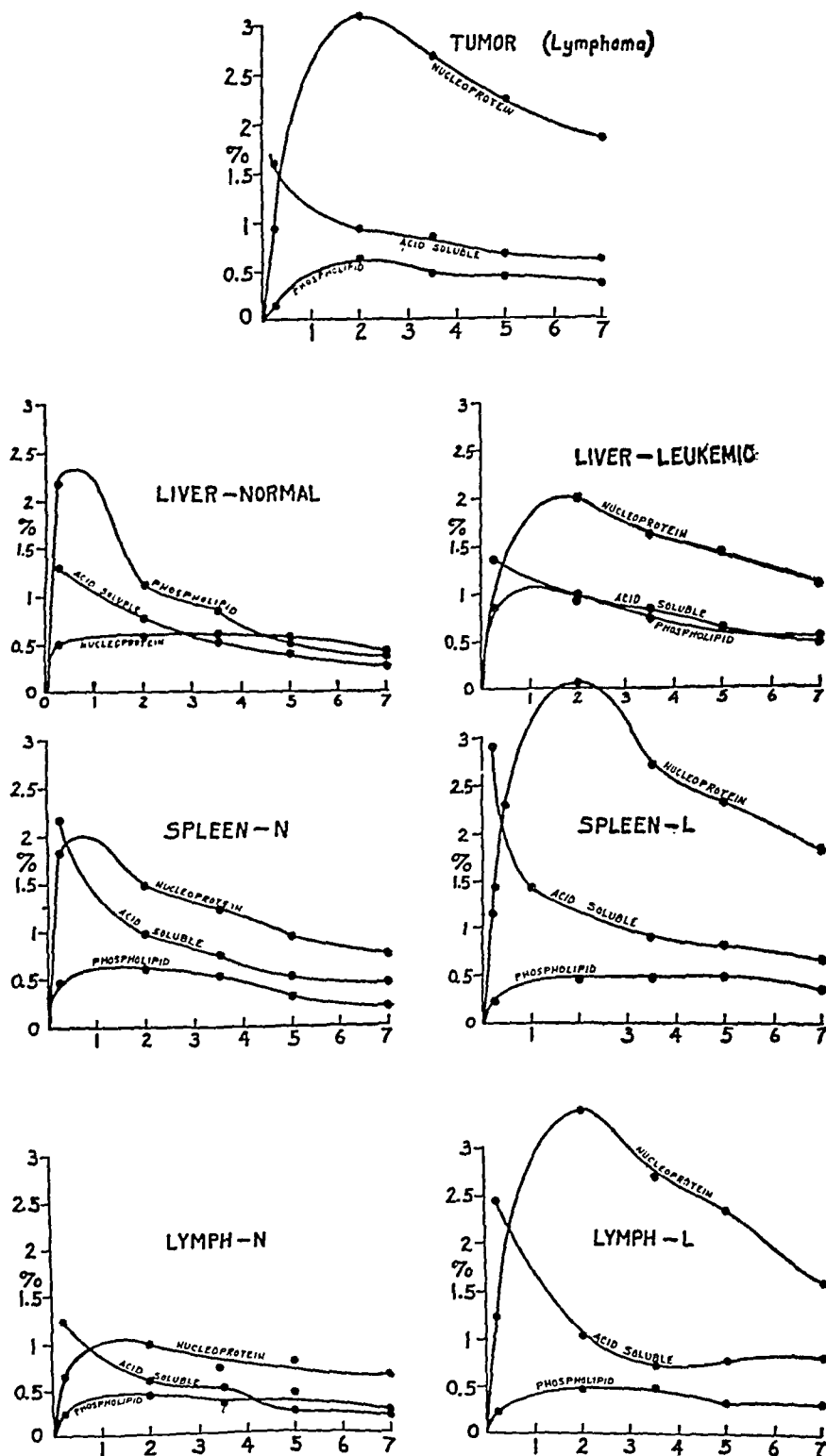


FIG. 1. THE COMPARATIVE UPTAKE OF LABELED-PHOSPHORUS IN NORMAL AND LEUKEMIC MICE

*B. Acid soluble.* The tissue residue from which the phospholipid had been removed was then extracted with four 70 cc. portions of cold, 5 per cent trichloroacetic acid. (Preliminary experiments had shown that the four extractions removed from 98 to 99 per cent of the activity which could possibly be extracted from the various tissues by this method.) Aliquot portions of the combined acid extracts were measured into ashing capsules for radioactivity and chemical analyses.

*C. Nucleoprotein.* The residual phosphorus contained in the tissues after the phospholipid and acid soluble fractions have been removed is termed "nucleoprotein" phosphorus although, in addition to nucleoprotein, this fraction probably contains some of the enzymes and co-enzymes (pyroadenylic acid, the pyridine nucleotides, flavine enzyme, etc.) involved in the intermediate transfer of phosphorus, in combination with their respective protein carriers. The "nucleoprotein" fraction, upon microscopic examination, is seen to consist mainly of free, partially fragmented nuclei, along with a smaller amount of amorphous cytoplasmic material.

Measurements of radioactivity were made by the use of a DuBridge type of ion chamber electrometer. Phosphorus analyses were made by the method of Pregl (4).

## RESULTS

The experimental data are presented in Table I.<sup>2</sup> Six hours after the administration of tagged phosphorus, the carcass of each control mouse retained an average of 71.8 per cent of the dose; while each leukemic carcass retained 70.3 per cent. At two days, each control retained 40.4 per cent, each leukemic 50.6 per cent; at 3½ days, control 38.5 per cent, leukemic 47.4 per cent; at 5 days, control 29.1 per cent, leukemic 41.5 per cent; at 7 days, control 25.1 per cent, leukemic 40.8 per cent.

The mean phosphorus contents of the nucleoprotein fractions derived from one gram of the various fresh tissues were as follows: Lymphoma 2.88 mgm. P/gram; leukemic liver 2.18 mgm./gram, normal liver 1.67 mgm./gram; leukemic spleen 2.95 mgm./gram; normal spleen 2.68 mgm./gram; leukemic lymph nodes 2.84 mgm./gram; normal lymph nodes 2.41 mgm./gram. For the acid soluble fractions the values were lymphoma 0.77 mgm. P/gram; leukemic liver 0.80 mgm./gram, normal liver 0.71 mgm./gram; leukemic spleen 0.83 mgm./gram, normal spleen 0.72 mgm./gram; leukemic lymph nodes 0.73 mgm./gram, normal lymph nodes 0.65 mgm./gram.

<sup>2</sup> Two other similar experiments, each involving over one hundred mice, gave practically identical results.

From Figure 1 it may be noted that for normal liver the activity curve of phospholipid is in keeping with a known function of liver in the synthesis of phospholipid. The curve indicates a very rapid incorporation of "tagged" phosphorus atoms into phospholipid and a rapid release of this phospholipid to other tissues. The relatively low, flat curve of nucleophosphorus activity in normal liver suggests that the turnover of nucleoprotein, insofar as phosphorus is concerned, is simply a metabolic phenomenon of tissue maintenance. No notably rapid synthesis of nucleoprotein or accelerated metabolism of the phosphorus transport systems is indicated for this tissue.

Normal spleen, which has a rôle in the destruction of the formed blood elements, and a possible function in the reorganization and resynthesis of their nucleoproteins, has a high order of nucleophosphorus activity indicative of intense nucleoprotein metabolism or synthesis. The phospholipid curve is low and relatively flat; one might infer that only the minimal exchange of phospholipid necessary for maintenance occurs.

Normal lymph nodes have a low level of phospholipid activity indicative of simple maintenance. The nucleophosphorus metabolism is intermediate between that of spleen and liver.

Lymphoma tissue is characterized by a very rapid incorporation of simple phosphate into nucleophosphate. The uptake is too rapid to be due to growth alone, and it seems reasonable to attribute it to some phase of phosphorus transport in glycolysis, which is a prominent metabolic feature of similar neoplasms of this type (5, 6).

After the infiltration of liver, spleen and lymphatic tissue by leukemic cells, a three- to fourfold increase is noted in the level of nucleoprotein activity. The activity curves of these tissues, in effect, tend to approach those of the pure lymphoma. The specific activity (activity per unit of phosphorus) in the nucleoprotein fraction of infiltrated liver, spleen and lymph nodes is several-fold higher than that of the corresponding normal tissues, although the phosphorus content of the leukemic tissues is only slightly increased over normal.

The curves of acid soluble activity for all the tissues show a sharp drop during the first few hours after the administration of the labelled phos-

phorus, followed by a gradual decrease over a period of several days. The acid soluble activity curve for each leukemic tissue is maintained at a considerably higher level than that of its respective normal tissue. Since these acid soluble fractions contain the phosphorus esters of carbohydrates undergoing glycolysis, along with the free intermediate carriers (adenylic acid, creatine phosphate, and the various nucleotides), which are part of the phosphorylating mechanisms, a reasonable explanation for the observed increase in activity would be that the quantity of these intermediates is increased as a result of leukemic infiltration or that the rate at which the compounds exchange phosphorus in their metabolic cycles is increased. Since the specific activity, or activity per unit weight of phosphorus is higher in the acid soluble fraction of leukemic tissue than in normal, the latter view has greater weight.

The phospholipid curves for infiltrated spleen and lymph nodes are little different from normal. The effect of infiltration upon liver phospholipid appears to be limited to a depression of the "synthesis peak" (7); the curve after two days is only slightly higher than normal.

Preliminary experiments on the phosphorus metabolism of yeast cells (*S. cerevisiae*) have shown that under fermentative conditions tagged phosphorus added to the external medium is very rapidly absorbed and converted to nucleophosphate. This, along with the rapid nucleophosphate exchange observed in tumor tissue, lends credence to the possibility that, in cells showing a high rate of carbohydrate cleavage, the phosphorylation of the intermediate phosphorus carriers involves their passage through the broad group of compounds which might fall under the generic term "nucleoprotein."

Further work to clarify the speculative points suggested by the findings of the present paper is in progress. A detailed study of the organic phosphorus compounds contained in the acid soluble and nucleoprotein fractions in the presence of selective toxins will be given later.

#### SUMMARY AND CONCLUSIONS

1. The manner in which a single tracer dose of radioactive phosphorus is handled over a period

of seven days by the tissues of fifty normal and fifty leukemic mice has been determined.

2. Leukemic infiltration is accompanied by an increase of several-fold in the uptake and retention of radioactive phosphorus by the nucleoprotein and acid soluble fractions of mouse liver, spleen and lymph nodes.

3. The phospholipid metabolism of spleen and lymph nodes is affected to only a limited extent by leukemic infiltration, while that of liver is depressed only during the first day after the administration of tagged phosphorus.

4. The whole body retention of phosphorus administered to leukemic mice is higher than that of normal mice, but the rate of excretion over a period of seven days is approximately the same.

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# MEASUREMENT OF THE EFFECT ON THE PAIN THRESHOLD OF ACETYSALICYLIC ACID, ACETANILID, ACETOPHENETIDIN, AMINOPYRINE, ETHYL ALCOHOL, TRICHLORETHYLENE, A BARBITURATE, QUININE, ERGOTAMINE TARTRATE AND CAFFEINE: AN ANALYSIS OF THEIR RELATION TO THE PAIN EXPERIENCE

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Analysis of the analgesia induced by the opiates has shown that these agents are capable not only of greater pain threshold-raising action than all other substances investigated, but also of important effects on the reaction to pain (1). Although the action of the non-opiate analgesics has been explored (2 to 20), the evidence hitherto presented is conflicting, often because the data have been non-quantitative, inadequate, or irrelevant. The development of a new method (21) for assaying quantitatively the effects of analgesic agents on man has made possible the following presentation of new data concerning the non-opiate analgesics.

## METHOD

The method employed here was described in detail in earlier studies on pain and analgesia (21). The light from a 1000 watt lamp was focussed by a condensing lens through a fixed aperture onto the blackened forehead of the subject. The surface of the forehead to be tested was thoroughly blackened with India ink. The intensity of the radiation was controlled by means of a rheostat. Immediately in front of the lamp was mounted an automatic shutter which was arranged to allow the radiation to pass through to the subject for exactly 3 seconds. The method of making the measurement of pain threshold was as follows: The subject seated himself and placed his forehead in position. The aperture was arranged so that 3.5 cm.<sup>2</sup> of blackened skin could be exposed. After a minute or so the shutter was lowered and the radiation allowed to fall on the skin for 3 seconds. The subject reported on his sensation. If no pain was experienced, the intensity of the light was increased and after 30 to 60 seconds the test was repeated. This procedure was followed until the subject just felt pain at the end of the exposure. This threshold pain was easily recognizable even by untrained subjects. The sensation was that of heat finally "swelling" to a distinct, sharp stab of pain at the end. When this condition had been reached the radiometer was placed in the aperture in place of the forehead of the subject and the

intensity of the radiation measured in gm. cal./sec./cm.<sup>2</sup> This value was considered to be the minimum stimulus for pain. The time required to make a single observation was usually less than 2 minutes. The maximum variation from the mean was in all cases less than 12 per cent, and several measurements agreeing within 2 per cent were made to establish a threshold.

After the control measurements, an analgesic agent was administered and observations of the pain threshold were made at 10 to 20-minute intervals until the threshold had returned to the control level. The height of the pain threshold-raising effect was expressed in per cent elevation above the control level. The protocols were distributed so that the threshold of one subject was measured by another subject who in turn was unfamiliar with the change in his own threshold. Occasionally, observers not participants in the experiment made threshold readings. Thus, three independent protocols were made, no individual knowing how much his own threshold had been altered.

With each pain threshold reading the subject made a concise statement of his psychological state. In the 10-minute interims between readings the subjects sat comfortably and engaged in reading, writing, or conversation. Sleep was not permitted and, if drowsy, the subjects walked about the laboratory. During long experiments food was taken, the three subjects consuming about the same amount of milk and bread. This required less than 15 minutes, after which the readings were continued.

In this series, all agents were given by mouth, with the exception of trichlorethylene, which was inhaled, and ergotamine and caffeine, which were injected intramuscularly. Therefore, the onset of action and the percentage increase in threshold and duration of action were variable. The subjects were, as in the earlier experiments, the three authors, each of whom weighed approximately 65 kilos.

The time-action curves for all agents in the amount given, were analyzed in three ways: (1) The maximum height of the pain threshold-raising effect; (2) the length of the period of effectiveness, and (3) the total threshold-raising action of the agent.

The maximum height of the threshold-raising action was obtained from the highest part of each time-action curve. The length of the effective period was taken to be the elapsed time between the administration of the

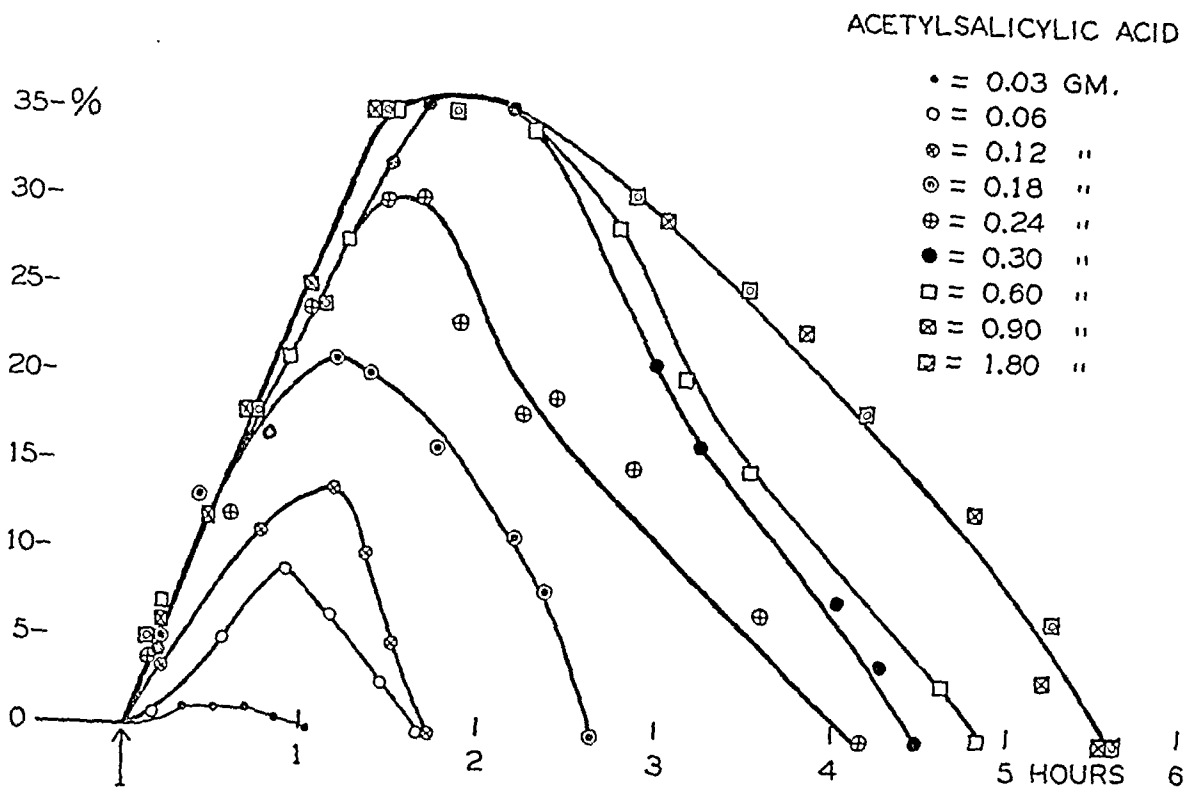


FIG. 1. TIME-ACTION CURVES FOR ACETYSALICYLIC ACID

The ordinate = per cent elevation of pain threshold over the control level as zero. The abscissa = duration of effect. The arrow indicates the time of ingestion of the acetylsalicylic acid. Each point represents the average of the threshold levels of two or three subjects.

agent and the return of the threshold to the pre-administration level. The total threshold-raising action was computed by multiplying the duration in hours of effect by the average per cent above the control level.

*Acetylsalicylic acid*

*Observations.* Twenty-four experiments were done with amounts from 0.03 gram to 1.8 grams of acetylsalicylic acid. There were at least two participants in each experiment and a total of 54 series of observations was made. The time-action curves for the nine different amounts studied were of a simple type (Figure 1).

The height and duration of the threshold-raising effect increased with the amount administered from 0.06 gram to 0.3 gram. Whether the amount of the agent was large or small the pain threshold was observed to rise within 10 to 15 minutes of ingestion and continued to rise at different rates until the peak effect for the particular amount had been reached.

*Comment.* It will be seen (Figure 2) that the maximum analgesic effect is directly proportional to quantities from 0.06 gram to 0.3 gram but is not increased with greater amounts. Further

threshold-raising effects might be obtained with very large quantities of this agent, but these are beyond the scope of this study.

The duration of the threshold-raising effect increased with the amount given from 0.06 gram to 0.9 gram (Figure 3), but the rate of increase became progressively smaller.

The relation of total threshold-raising action to amount is shown in Figure 4. There was an increase in effect with the amount up to 0.3 gram. With each additional 0.3 gram from 0.3 to 0.9 gram there was an increase of about 10 per cent, but doubling 0.9 gram, or 1.8 grams, increased the effect only slightly.

*Observation.* The time-action curves (Figure 1) indicate that the total threshold-raising effect realized from a given amount of acetylsalicylic acid will depend upon the frequency of dosage. Acetylsalicylic acid in 0.3 gram amounts was administered orally at 2-hour intervals over a period of 8 hours. The readings recorded at 10- to 20-minute intervals throughout this period show that, following the first 0.3 gram amount, the threshold was raised to the usual level, after which the pain threshold remained elevated for about 6 hours

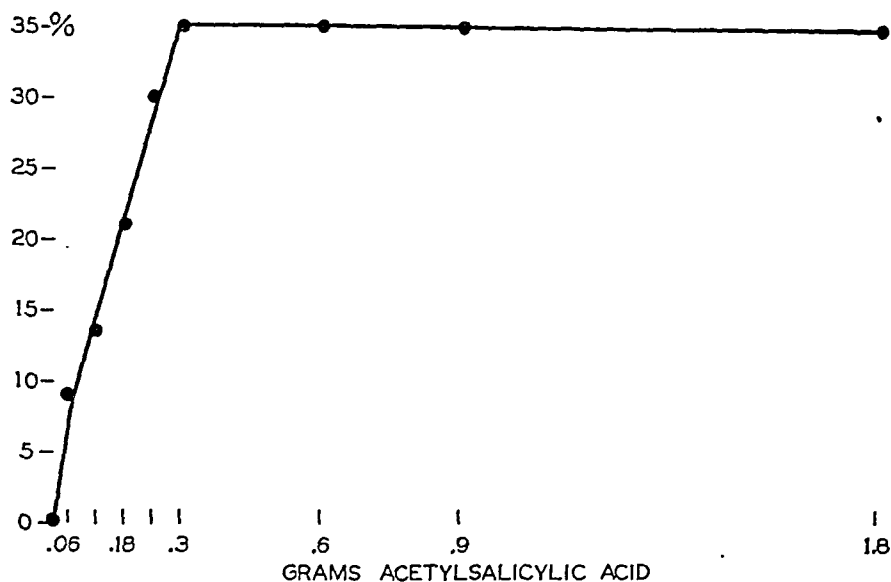


FIG. 2. THE MAXIMUM PAIN THRESHOLD-RAISING EFFECT OF ACETYLSALICYLIC ACID FOR QUANTITIES FROM 0.06 GRAM TO 1.8 GRAMS

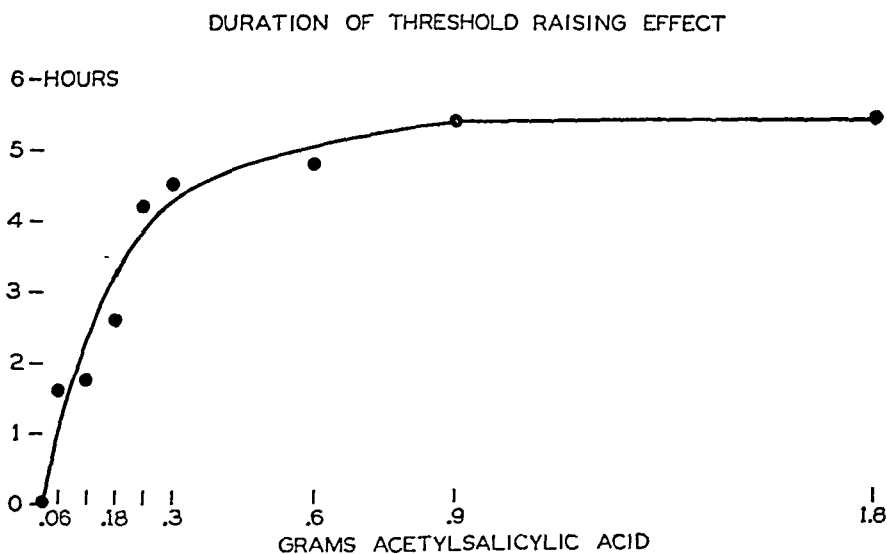


FIG. 3. THE RELATION BETWEEN DURATION OF THRESHOLD-RAISING EFFECT (ORDINATE) AND THE QUANTITY OF ACETYLSALICYLIC ACID ADMINISTERED (ABSCISSA)

at 30 to 35 per cent above the control level (Figure 5).

In contrast 0.6 gram of acetylsalicylic acid given three times at intervals of 3 hours resulted in unstable elevation of the pain threshold (Figure 6).

*Comment.* Comparison of Figures 5 and 6

demonstrates that 1.2 grams of acetylsalicylic acid administered in amounts of 0.3 gram every 2 hours produce at least a third more threshold-raising effect in 8 hours than 1.8 grams administered in amounts of 0.6 gram every 3 hours. From the standpoint of therapy, these experiments indicate that it is desirable to give frequent administra-



## TOTAL THRESHOLD RAISING EFFECT

120-HOURS PERCENT

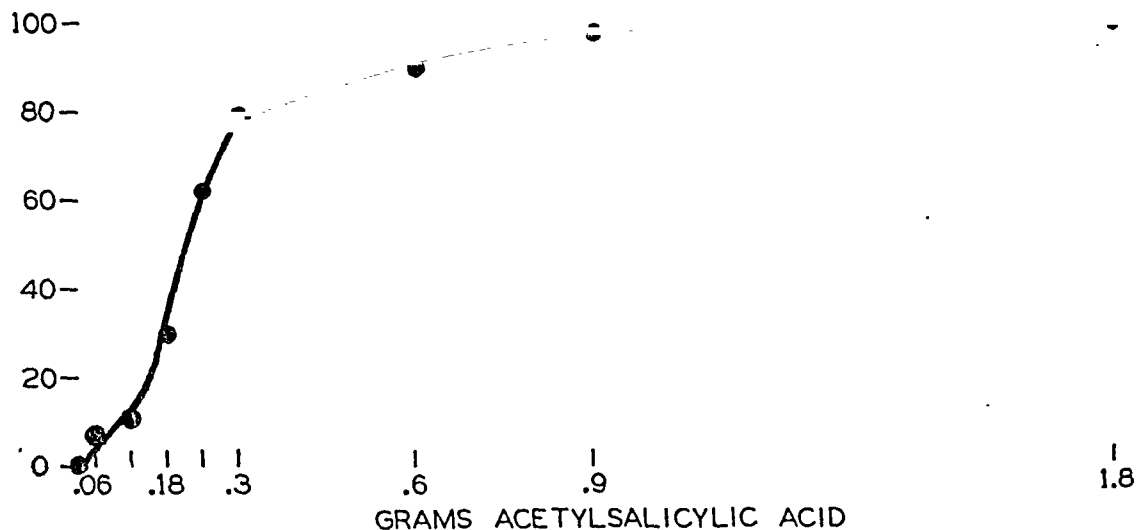


FIG. 4. THE RELATION BETWEEN THE TOTAL THRESHOLD-RAISING EFFECT AND THE QUANTITY OF ACETYLSALICYLIC ACID ADMINISTERED

The ordinate was computed by multiplying the average per cent rise in pain threshold by the hours of duration of effect resulting from a given quantity of acetylsalicylic acid. The abscissa = quantity of acetylsalicylic acid administered.

REPEATED ADMINISTRATIONS OF  
ACETYLSALICYLIC ACID  
0.3 GM.

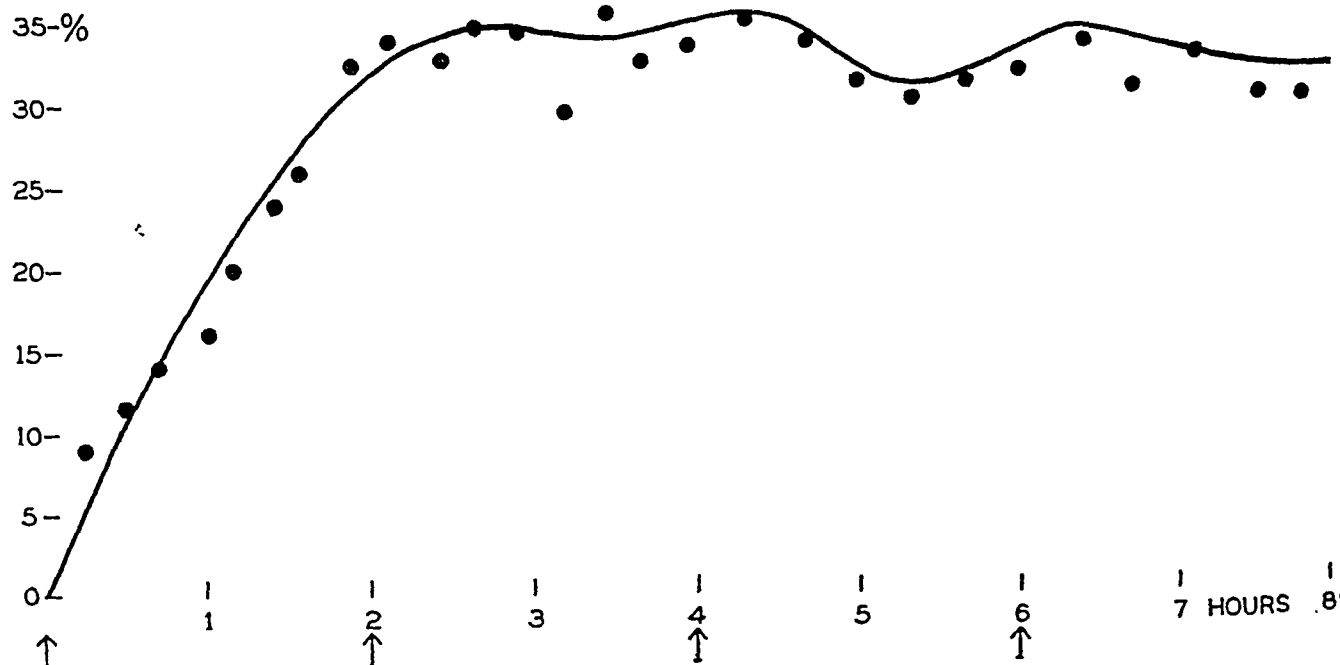


FIG. 5. THE EFFECT ON THE PAIN THRESHOLD OF REPEATED INGESTIONS OF ACETYLSALICYLIC ACID 0.3 GRAM AT 2-HOUR INTERVALS

Each point represents the average of the threshold levels of two subjects.

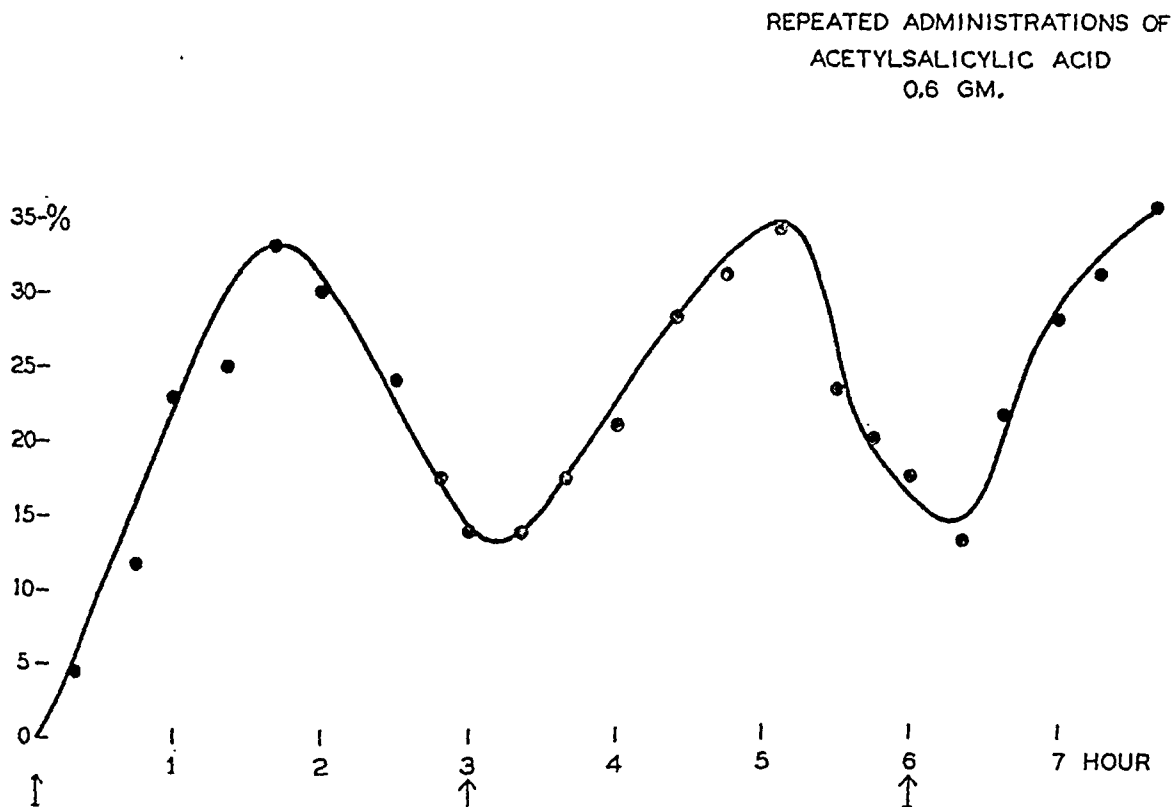


FIG. 6. THE EFFECT ON THE PAIN THRESHOLD OF REPEATED INGESTIONS OF ACETYSALICYLIC ACID 0.6 GRAM AT 3-HOUR INTERVALS

Each point represents the average of the threshold levels of two subjects. The arrows indicate the time of ingestion of the acetylsalicylic acid.

tions of acetylsalicylic acid, since the essential elimination of this agent is very rapid. Secondly, Figures 5 and 6 demonstrate that in no case is a threshold-raising effect of much more than 35 per cent above the control level realized. This gives added support to the conception of a saturation amount; that is, effective addition of threshold-raising action occurs only for quantities less than the saturation amount of 0.3 gram. Similar "saturation effects" were observed in studies on codeine phosphate (1).

*Psychological and other effects. Observations.* Acetylsalicylic acid in amounts as small as 0.18 gram had a mild sedative effect as well as pain threshold-raising action. The sedative effect remained slight even with amounts ten times larger, i.e. 1.8 grams. The three subjects experienced mild relaxation and lethargy. Little or no effect on anxiety or tension was noted except that restlessness was reduced. There were no induced feelings of contentment, euphoria, or apathy. No

untoward effects resulted except slight burning sensations in the epigastrium after larger amounts, and slight difficulties in attention and concentration after repeated administrations of the agent at 2-hour intervals.

*Comment.* After the administration of acetylsalicylic acid the subject, during successive threshold readings, became aware of a change in the character of the stimulus. The sensation of heat which preceded the onset of pain became relatively more intense and it was with surprise that no pain was experienced. The probable explanation of this phenomenon is that the threshold of heat is actually lowered by acetylsalicylic acid (21). No such experience followed the administration of opiates.

#### *The effect of pain on the action of acetylsalicylic acid*

It has previously been shown that the threshold-raising action of the opiates is considerably modi-

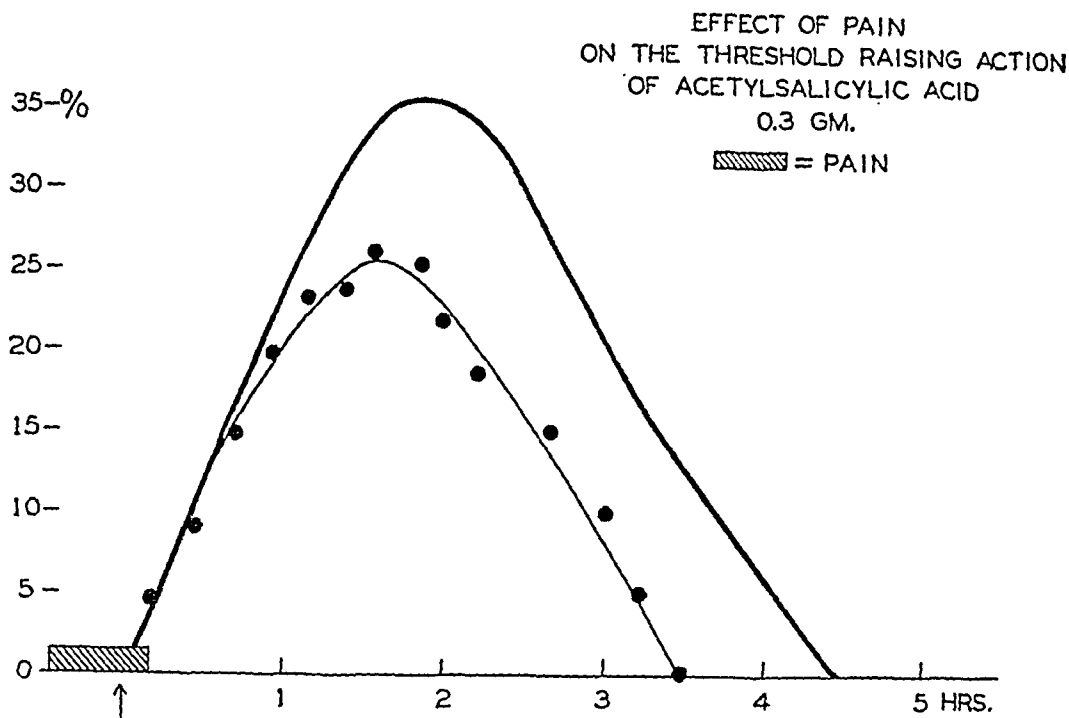


FIG. 7. THE EFFECT OF SUSTAINED PAIN FOR 40 MINUTES ON THE PAIN THRESHOLD-RAISING PROPERTIES OF ACETYSALICYLIC ACID (0.3 GRAM)

The heavy black line represents the pain threshold-raising effect of 0.3 gram acetylsalicylic acid in three subjects without pain. The light line represents the pain threshold-raising effect of 0.3 gram acetylsalicylic acid when given 27 minutes after the beginning of pain. Each curve represents the average of the threshold levels in two subjects. The arrow indicates the time of administration. The ▨ indicates the duration of the pain.

fied by pain introduced just before or soon after their administration (1). Indeed, pain of moderate intensity of 40 minutes' duration seriously reduces the threshold-raising action of 0.015 gram of morphine. It has been pointed out that the therapeutic usefulness of the opiates, therefore, depends largely upon qualities other than those which raise the pain threshold (1).

Acetylsalicylic acid differs from the opiates also in this regard.

*Method.* Experiments using induced pain, identical with those done with the opiates, were repeated with acetylsalicylic acid. A sphygmomanometer cuff placed about the arm was inflated to 220 mm. Hg and then kept in place for 40 minutes. During this period there gradually developed increasingly intense pain. Ten to 15 minutes before the termination of the pain period, acetylsalicylic acid was administered orally in amounts of 0.3 gram to 0.9 gram.

*Observations.* Pain experienced during the first part of the action of the acetylsalicylic acid reduced the threshold-raising effect and the dura-

tion of action of the agent in three experiments representing seven observations. In Figure 7 is shown the effect of pain on the threshold-raising action of 0.3 gram. In one experiment 0.9 gram was taken instead of 0.3 gram. The threshold-raising action of both amounts of the drug was affected to about the same degree by the pain.

*Comment.* Without a large series of experiments performed under various circumstances, no final statement of the influence of pain on the effects of acetylsalicylic acid can be made, but presumably pain may cause the agent to lose about a quarter of its effectiveness. The amount of effect of the pain probably depends on many factors, including duration and intensity of pain, but these have not been analyzed. The effect of pain on the action of acetylsalicylic acid is in striking contrast to what occurs when pain precedes or overlaps the administration of morphine (1). Here most of the threshold-raising effect may be eliminated by an intensity and duration of pain no greater than that employed here.

*Acetylsalicylic acid as a therapeutic agent*

The ability to raise the pain threshold coupled with relatively minor effects on mood or mentation gives acetylsalicylic acid, and presumably other salicylates, a unique position in therapy. It may be administered in relatively large amounts over long periods with slight danger of dependence.

Acetylsalicylic acid has been found to be especially useful in pains of low intensity, not only when circumscribed but also when widespread in origin. It is not the total distress experienced by the patient, but the intensity of the pain which determines the therapeutic effectiveness of a given threshold-raising agent. If the pain is of an intensity that cannot be abolished or greatly reduced by the threshold-raising effect of the agent, then no matter how limited the area or distribution is, no amount of this substance will stop the pain. For example, the intense pain associated with coronary occlusion, though it emanates from a very limited area, is not eliminated by acetylsalicylic acid. On the other hand, the relatively low-grade pain of rheumatic fever, though very widespread in origin, is dramatically abolished.

These facts are compatible with the evidence concerning non-spatial-summation of pain formulated elsewhere (21). Thus the total intensity of several areas of different intensities of pain is equal, not to their sum, but merely to the intensity of the severest pain. It requires no more acetylsalicylic acid to abolish a widespread area of low-grade pain than it does to abolish a very small area of pain of the same intensity.

Apparently a total of 3.6 grams per day taken in divided doses of 0.3 gram every 2 hours is more effective than a total of 4.8 grams per day taken in divided doses of 0.6 gram every 3 hours. Since the actions of 0.3 gram and 0.9 gram amounts of acetylsalicylic acid were about equally affected by pain, there is little evidence to suggest that the ingestion of larger amounts would be more effective than the 0.3 gram amount in the presence of pain.

There is the possibility that quantities of acetylsalicylic acid greater than those necessary to raise the pain threshold the maximal amount may be required to attain other useful actions of this agent.

*Acetylsalicylic acid in combination with other agents*

Three experiments, representing 8 series of observations, were done with combinations of codeine phosphate and acetylsalicylic acid. First, a combination of amounts of these agents having about the same threshold-raising effect was administered (1). Acetylsalicylic acid was taken by mouth in 0.3 gram amounts and 30 minutes later 0.03 gram of codeine phosphate was given hypodermically.

The threshold-raising effect of this salicylate and codeine combination under these conditions was no greater than the action of each taken separately (Figure 8a). The sedative and mood-changing effects of the codeine were well defined.

In another experiment, 0.6 gram of acetylsalicylic acid was taken by mouth, and one hour later 0.03 gram of codeine phosphate was injected intramuscularly. A threshold-raising effect of approximately 35 per cent resulted, showing that the addition of codeine had no further threshold-raising action (Figure 8b).

To ascertain whether the threshold-raising effect of 0.06 gram of codeine could be raised above its saturation level of 45 to 50 per cent above threshold level, 0.9 gram of acetylsalicylic acid was combined with codeine. The codeine was administered intramuscularly 27 minutes after ingestion of the acetylsalicylic acid. The combination had a threshold-raising effect but slightly different from that of 0.06 gram of codeine alone (Figure 8c).

The combination of acetylsalicylic acid and calcium was investigated. Three-tenths gram of acetylsalicylic acid and 2 grams of calcium gluconate were taken by mouth. The threshold-raising effect and duration of action that resulted from the combination were no greater than that obtained from 0.3 gram of acetylsalicylic acid alone.

*Comment.* It has thus been shown that there is no addition of pain threshold-raising effect when codeine and acetylsalicylic acid are taken together in various amounts.

Nevertheless, the therapeutic usefulness of such a combination may not be waived. Codeine, as a typical opiate, has two important actions (1): (1) It impedes to a greater or lesser degree the perception of pain; and (2) it detaches the perception of pain from the fight-flight-anxiety reaction so

stable pain threshold-raising effects of the salicylates lends a therapeutic usefulness when the two aforementioned effects are desirable. It is evident, however, that as regards pain threshold-raising effects no addition accrues from this combination.

### *Acetanilid*

Five experiments were done with 0.3 gram of acetanilid. A total of 12 series of observations was thus available for analysis (Figure 9). The height and duration of the threshold-raising effect were of the same order as that of acetylsalicylic acid. Relaxation, drowsiness and difficulty in mentation were noted, and concentration, retention and attention were all slightly impaired. Restlessness was allayed and even anxiety was diminished, but there was no euphoria.

*Effect of pain on the action of acetanilid.* As in the case of acetylsalicylic acid, the threshold-raising effect and duration of action were both disturbed if the initial action of the agent was

coupled with pain (Figure 10). Here, too, however, the destructive action of the pain was relatively slight.

### *Acetophenetidin*

Two experiments with 0.3 gram of acetophenetidin representing 6 series of observations were made (Figure 11). Psychological effects from acetophenetidin were more pronounced than after acetylsalicylic acid and similar to those produced by acetanilid.

*Comment.* It is possible that these psychological effects of acetanilid and acetophenetidin coupled with their threshold-raising action are responsible for the popularity of these agents as headache remedies, especially where tension or anxiety is dominant.

### *Aminopyrine*

Aminopyrine was given in 0.3 gram amounts in one experiment representing 3 series of observations. The maximum threshold-raising effect averaged about 31 per cent above the control level

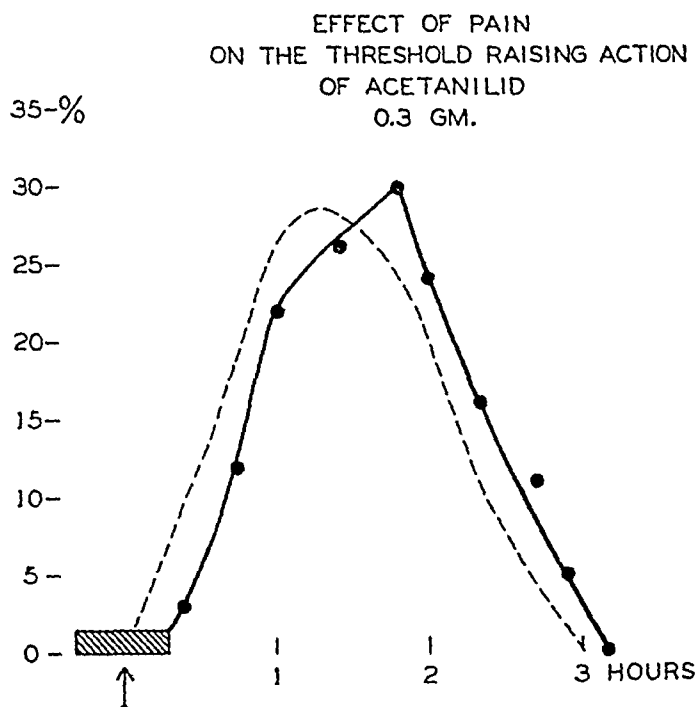


FIG. 10. THE EFFECT OF SUSTAINED PAIN (40 MINUTES) ON THE PAIN THRESHOLD-RAISING PROPERTIES OF ACETANILID

The dashed line represents the pain threshold-raising effect of 0.3 gram acetanilid without pain. The solid line represents the effect with pain (average for three subjects). The arrow indicates the time of oral administration of the acetanilid. The   indicates the duration of the pain.

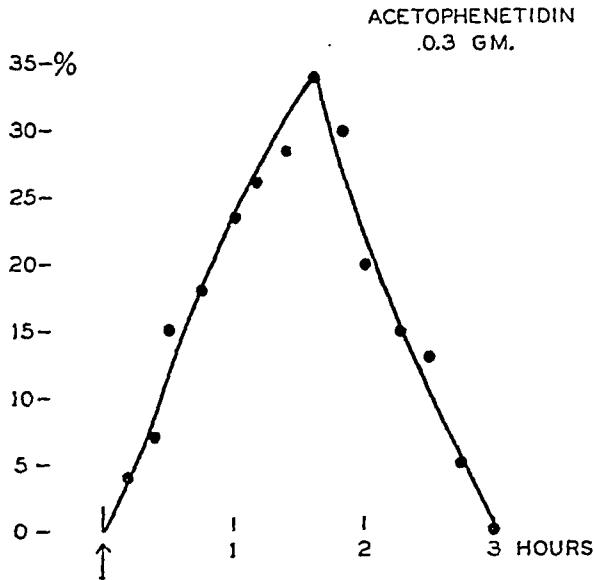


FIG. 11. THE AVERAGE PAIN THRESHOLD ELEVATION FOR THREE SUBJECTS RESULTING FROM THE ADMINISTRATION OF 0.3 GRAM ACETOPHENETIDIN

The arrow indicates the time of oral administration.

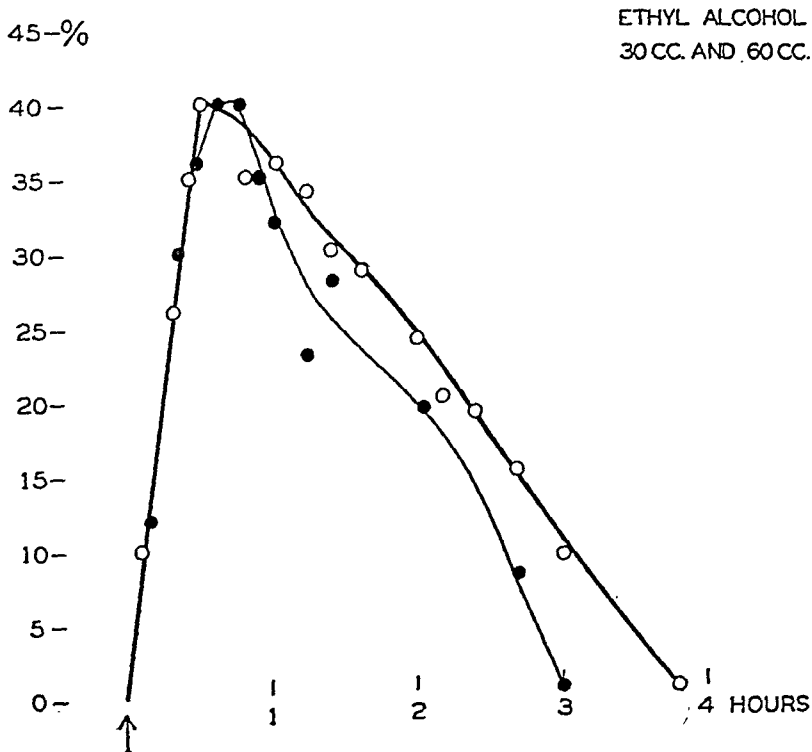


FIG. 12. THE PAIN THRESHOLD ELEVATION RESULTING FROM THE ORAL ADMINISTRATION OF 30 CC. AND 60 CC. OF ETHYL ALCOHOL, 95 PER CENT

Each solid dot represents the average of the threshold levels in three subjects after 30 cc. of alcohol; the hollow dots, the same, after 60 cc. of alcohol. The arrow indicates the time of administration of the alcohol.

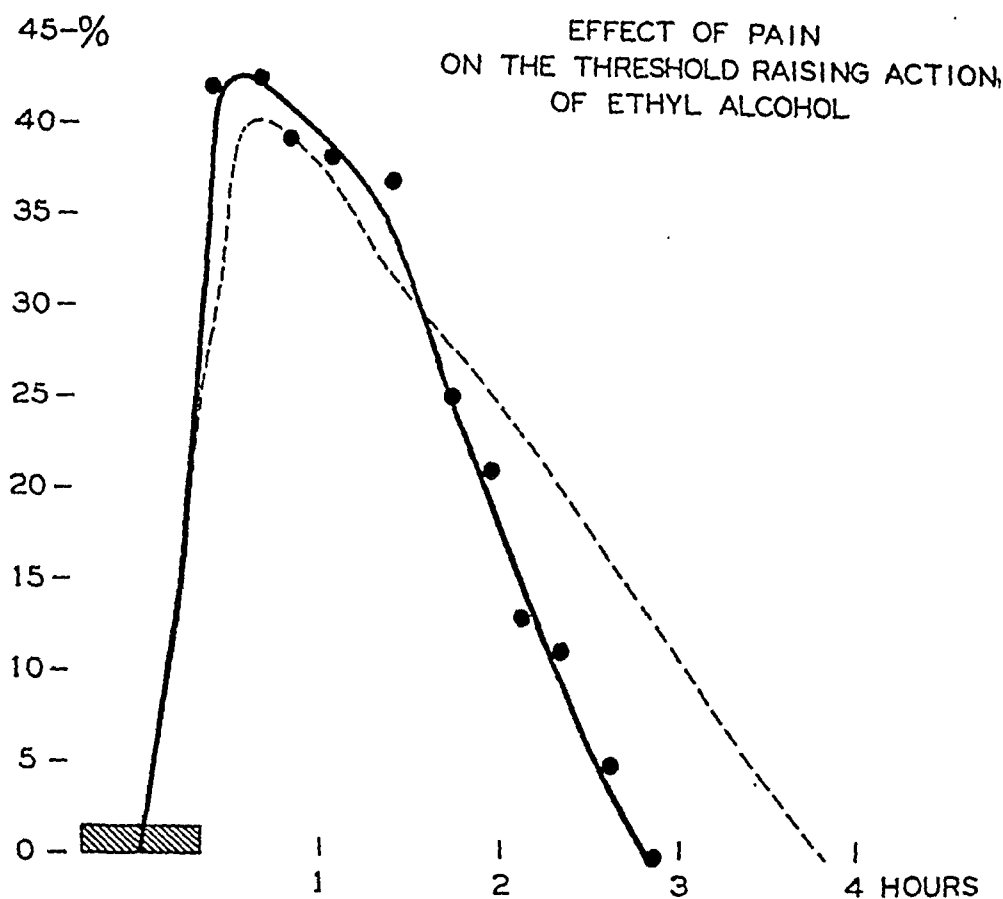


FIG. 13. THE EFFECT OF SUSTAINED PAIN (40 MINUTES) ON THE PAIN THRESHOLD-RAISING PROPERTIES OF ETHYL ALCOHOL (60 CC., 95 PER CENT) ADMINISTERED 20 MINUTES AFTER THE BEGINNING OF PAIN (SOLID LINE), AVERAGE OF THREE SUBJECTS

The dashed line represents the pain threshold-raising effect of 60 cc. of ethyl alcohol in three subjects without pain. The ||||| indicates the duration of the pain.

and was attained about one hour after ingestion. The threshold remained elevated at or near the maximum for 30 minutes. The threshold was elevated above the control level for about 2 hours. With the exception of very slight relaxation, no other effects were noted.

#### *Ethyl alcohol*

Four experiments representing a total of 12 series of observations were done. Two of these were with amounts of 30 cc. of 95 per cent alcohol, and two with 60 cc. (Figure 12). The alcohol was ingested about 3 to 5 hours after the last meal and all of it was swallowed within 2 minutes. The alcohol was diluted with charged water containing sugar for sweetening so that the total volume of fluid was 250 cc.

*Psychological and other effects.* Alcohol in these subjects produced changes important because of their effect on pain, namely, the induced

feelings of relaxation, contentment, detachment, and euphoria. Also, time appreciation was altered so that standard intervals seemed shorter. Concentration, attention and retention were defective, memory for events that occurred during this phase was blurred, and restraint, judgment and discrimination were impaired. This period of euphoria lasted about an hour and was followed by one of depression and lethargy. Although the mood had changed, the defects in concentration, attention and retention persisted for another 2 hours.

The peak of the threshold-raising effect and the peak of excitement and euphoria occurred at about the same time. The period of increasing depression and lethargy was associated with fall of the pain threshold to the normal control level.

*Effect of pain on the threshold-raising action of ethyl alcohol.* Experiments identical with those done with the opiates, acetylsalicylic acid, and acetanilid were performed with alcohol. Pain of

40 minutes' duration was induced by the tight sphygmomanometer cuff about the arm. The alcohol was imbibed about 20 minutes after the cuff had been inflated and when pain was already very intense. Neither the height nor duration of the threshold-raising effects of the alcohol was substantially reduced as a result of the pain (Figure 13).

The subjects were able to perceive pain of high intensity, but within 3 minutes of the time of ingestion the reaction to the pain began to change to one of detachment. The reaction to pain was altered in a manner not unlike that experienced after morphine, so that, although the subject perceived pain, he became indifferent to it. Restlessness and pacing of the floor because of the pain from the tight cuff were lessened within 5 minutes of ingestion.

*Comment.* Ethyl alcohol thus had a double effect not unlike morphine (1): (1) It raised the threshold in the presence or absence of pain, and (2) it changed the reaction to pain in such a way as to cause the subject to feel detached about such pain which he was perceiving at the time. Alcohol has a seeming superiority over morphine as a therapeutic agent since the latter has its threshold-raising action reduced or abolished by pain. But the overactivity and lack of restraint induced by the ethyl alcohol could be undesirable for the patient as well as for those about him.

### *Trichlorethylene*

The most swiftly acting of the analgesic agents investigated was trichlorethylene which, when inhaled, had its maximum effect even sooner than alcohol. Two experiments representing 4 series of observations were done. One cc. of trichlorethylene (Calco Chemical Co.) was inhaled under as uniform conditions as inhalation experiments permit. The pain threshold was measured on the head in the usual manner and on the back of the hand. Identical threshold-raising results were observed. The time required to reach the maximum elevation was approximately 15 minutes. The threshold remained elevated at or near the maximum for 9 to 20 minutes, depending on the speed of inhalation, and remained above the control level for about three quarters of an hour (Figure 14). During the inhalation, the subjects

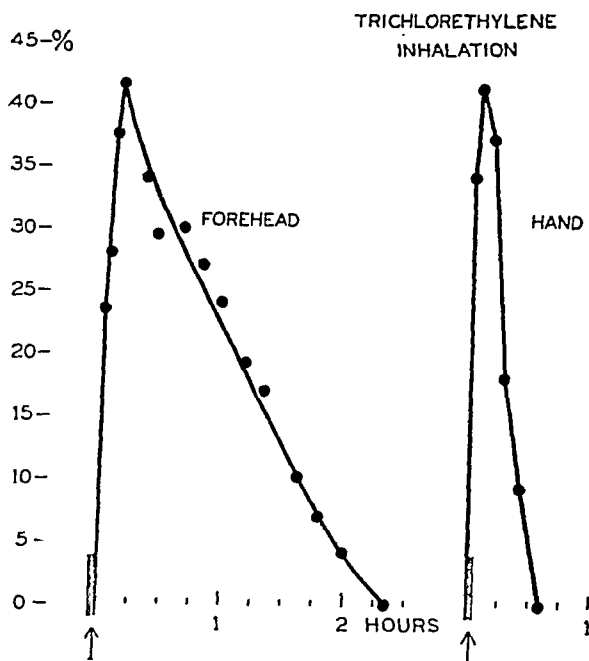


FIG. 14. THE AVERAGE PAIN THRESHOLD ELEVATION FOR THREE SUBJECTS RESULTING FROM THE ADMINISTRATION (INHALATION) OF TRICHCLORETHYLENE

Readings made on the forehead in the first experiment and on the back of the hand in the second experiment. The arrows indicate the periods (4 minutes) of inhalation of the contents of 1 cc. trichlorethylene "pearls."

experienced sensations of "light-headedness" and impending syncope. It is of interest that the threshold-raising effect was not limited to the head, but was equally great on the hand. This observation was made earlier (11) by the use of stiff hairs on pain endings in the skin.

### *Barbiturates*

One experiment representing 3 series of observations was done with 0.5 gram of the sodium salt of N-methylcyclohexenylmethyl barbituric acid ("Evipal" brand). This variety of barbiturate was selected because of its prompt action and elimination. By keeping the subjects awake, the threshold-raising effects due to sleep, *per se*, were avoided. As seen in Figure 15, an amount of barbiturate barely compatible with a waking state produced a threshold-raising effect of only 21 per cent.

Out of all proportion to the threshold-raising effect were the sedative and hypnotic effects. Within 20 minutes of ingestion the subjects be-



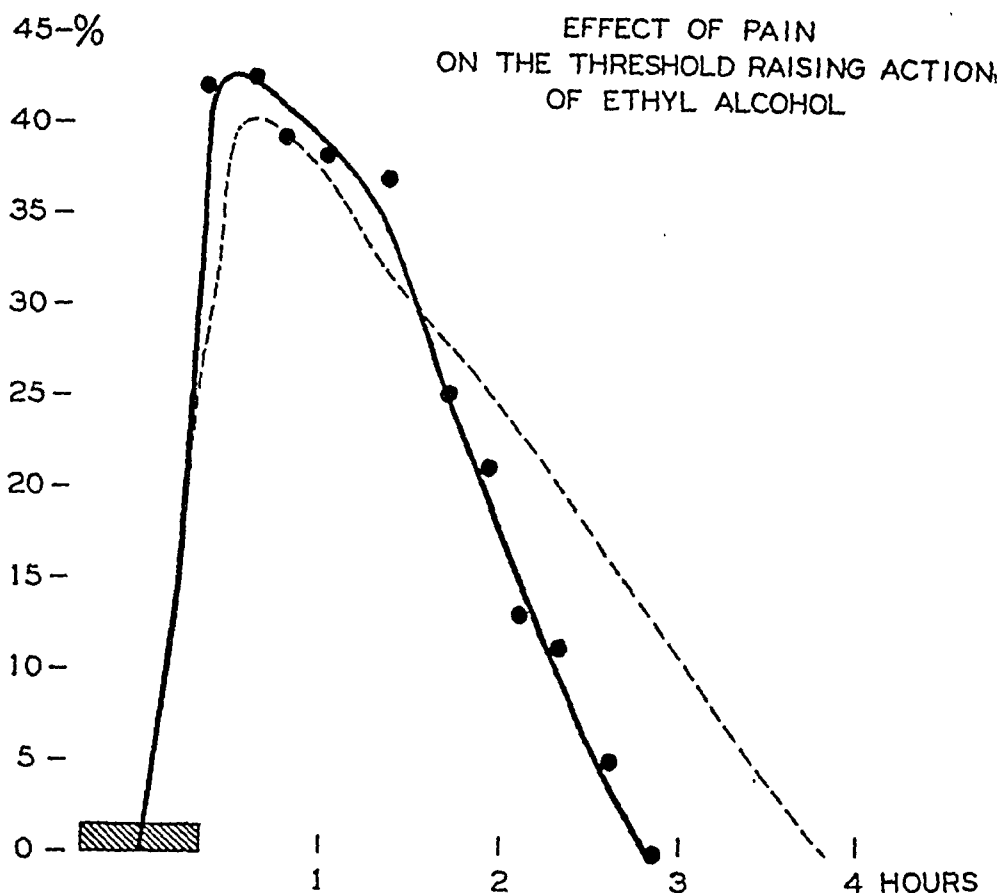


FIG. 13. THE EFFECT OF SUSTAINED PAIN (40 MINUTES) ON THE PAIN THRESHOLD-RAISING PROPERTIES OF ETHYL ALCOHOL (60 CC., 95 PER CENT) ADMINISTERED 20 MINUTES AFTER THE BEGINNING OF PAIN (SOLID LINE), AVERAGE OF THREE SUBJECTS

The dashed line represents the pain threshold-raising effect of 60 cc. of ethyl alcohol in three subjects without pain. The ||||| indicates the duration of the pain.

and was attained about one hour after ingestion. The threshold remained elevated at or near the maximum for 30 minutes. The threshold was elevated above the control level for about 2 hours. With the exception of very slight relaxation, no other effects were noted.

#### *Ethyl alcohol*

Four experiments representing a total of 12 series of observations were done. Two of these were with amounts of 30 cc. of 95 per cent alcohol, and two with 60 cc. (Figure 12). The alcohol was ingested about 3 to 5 hours after the last meal and all of it was swallowed within 2 minutes. The alcohol was diluted with charged water containing sugar for sweetening so that the total volume of fluid was 250 cc.

*Psychological and other effects.* Alcohol in these subjects produced changes important because of their effect on pain, namely, the induced

feelings of relaxation, contentment, detachment, and euphoria. Also, time appreciation was altered so that standard intervals seemed shorter. Concentration, attention and retention were defective, memory for events that occurred during this phase was blurred, and restraint, judgment and discrimination were impaired. This period of euphoria lasted about an hour and was followed by one of depression and lethargy. Although the mood had changed, the defects in concentration, attention and retention persisted for another 2 hours.

The peak of the threshold-raising effect and the peak of excitement and euphoria occurred at about the same time. The period of increasing depression and lethargy was associated with fall of the pain threshold to the normal control level.

*Effect of pain on the threshold-raising action of ethyl alcohol.* Experiments identical with those done with the opiates, acetylsalicylic acid, and acetanilid were performed with alcohol. Pain of

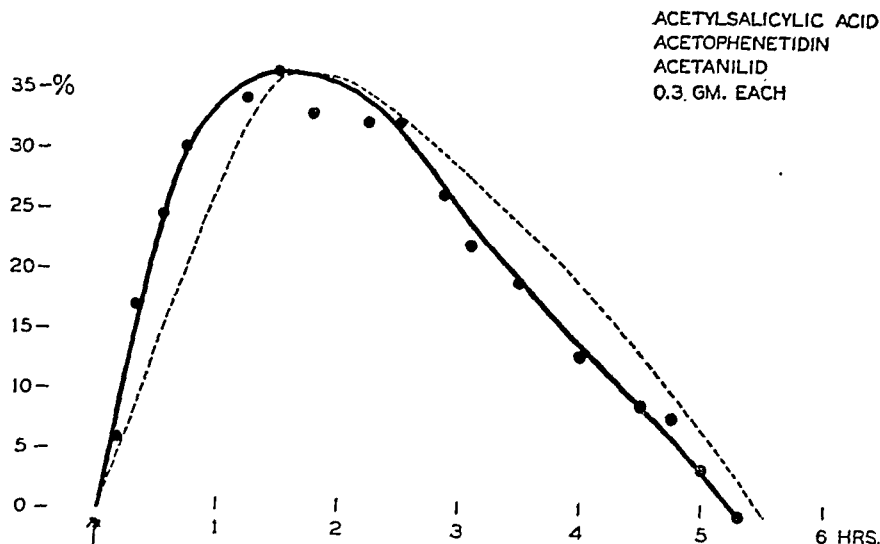


FIG. 16a. THE AVERAGE PAIN THRESHOLD ELEVATION FOR THREE SUBJECTS RESULTING FROM THE ADMINISTRATION OF THE COMBINATION OF ACETYSALICYLIC ACID, ACETOPHENETIDIN AND ACETANILID, 0.3 GRAM EACH (SOLID LINE)

The dashed line represents the pain threshold elevation resulting from the administration of 0.9 gram acetylsalicylic acid. The arrow indicates the time of oral administration of the agents.

#### *Ergotamine tartrate*

The dramatic termination of migraine headache by 0.5 mgm. injections of ergotamine tartrate aroused curiosity concerning the nature of the analgesic action of this agent. It has been demonstrated (22) that the decline in intensity of the headache is coincident with the decrease in amplitude of pulsations of the cranial arteries, chiefly the branches of the external carotid arteries. It was inferred that the contraction of dilated arteries reduced the deformity of pain end organs about these arteries and so terminated the headache.

In one experiment representing 3 series of observations, ergotamine tartrate (0.5 mgm. intramuscular) did not raise the pain threshold. It induced feelings of prostration with nausea. Therefore, such effect as this agent has in diminishing headache cannot be attributed to a pain threshold-raising action.

#### *Other combinations*

It is important in appraising the action of combinations to separate clearly the resultant pain threshold-raising action of a combination from other desirable effects. It is readily appreciated

that one may wish to combine an agent with good pain threshold-raising action with one having suitable sedative and hypnotic action, as well as other effects on mood and mentation.

*Observations.* The combination of acetophenetidin, acetanilid, and acetylsalicylic acid each in 0.3 gram amounts was tested once, representing 3 series of observations (Figure 16a). The combination had no greater threshold-raising action than an equivalent weight (0.9 gram) of acetylsalicylic acid. However, the sedative and hypnotic effect of the combination was far greater than the latter.

The threshold-raising effect of the well-known combination of caffeine citrate, 0.15 gram; acetophenetidin, 0.15 gram; acetylsalicylic acid, 0.15 gram, was investigated in one experiment, representing 3 series of observations. Two tablets, each containing the above mixture and amounts, were taken. The threshold-raising effect of the combination was no greater than an equal weight (0.9 gram) of acetylsalicylic acid. However, this combination, as well as the previous one, presented effects which were worthy of note. Relaxation, lethargy, and freedom from restlessness were well defined. It appeared to the observers that the

came profoundly relaxed, lethargic and unsteady on their feet. They were slightly loquacious. Concentration, attention and retention were seriously disturbed and manipulative skill was much reduced. Acts requiring discrimination were poorly done. The pain threshold-raising effects of this barbiturate in combination with acetanilid and acetylsalicylic acid are discussed below.

*Comment.* Under the circumstances of this experiment, this barbiturate had relatively slight analgesic action and, since it is in no way unrepresentative, all barbiturates probably behave somewhat similarly. However, if sleep is induced, an entirely new set of analgesic factors may be introduced. Sleep as a threshold-raising factor has not been fully studied, but initial experiments indicate considerable threshold elevation during sleep. When consciousness is maintained, barbiturates have poor pain threshold-raising action. Eddy previously came to a similar conclusion (6).

### Quinine

In one experiment representing 3 series of observations, 0.3 gram quinine sulphate taken by mouth did not raise the pain threshold above the control level.

### Caffeine

Because of its frequent use in so-called "head-ache mixtures," as well as other combinations of analgesics, the effect of caffeine salts on the pain threshold was ascertained. In one experiment representing 3 series of observations, caffeine sodiobenzoate in 0.5 gram (intramuscular) amounts had no measurable action on the threshold. Ten minutes after the administration of the caffeine the subjects began to experience a series of changes, only a few of which are relevant to this discussion. Significant were the feelings of alertness, well-being, and improved general effectiveness.

*Comment.* It is evident from these data that caffeine has no threshold-raising action. However, caffeine when taken by mouth has a slight vasoconstrictor action on the cranial arteries (23) and may thus be a factor in relieving certain headaches in a manner akin to, but far less effectively than, ergotamine tartrate (22, 23). The notion that it improves circulation and offsets the detrimental action of the pyrazoline compounds is little supported by bedside or experimental evidence. The mild, induced exhilaration above described may engender this impression.

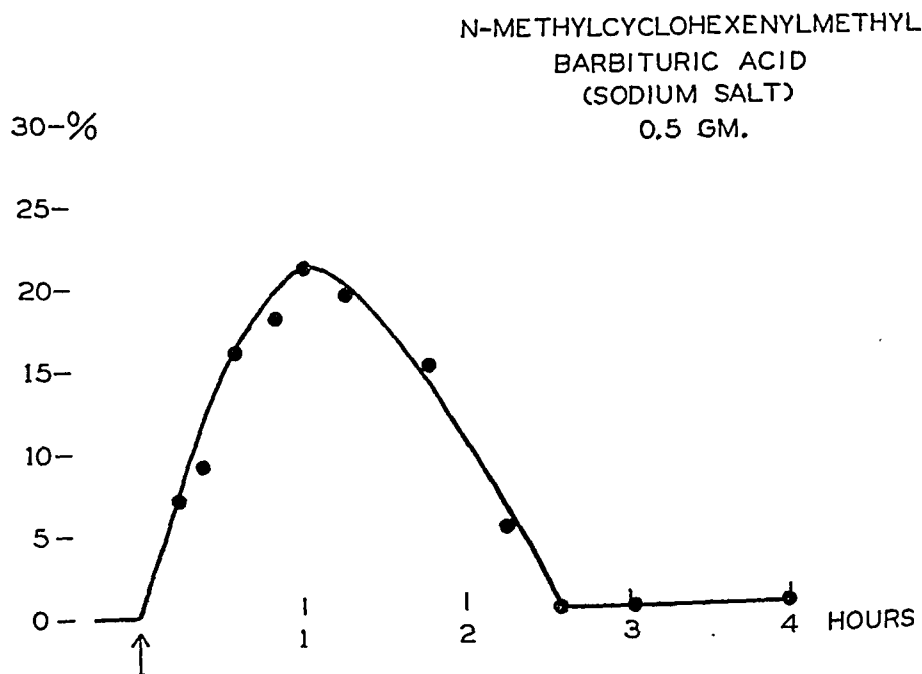


FIG. 15. THE AVERAGE PAIN THRESHOLD ELEVATION FOR THREE SUBJECTS RESULTING FROM THE ORAL ADMINISTRATION OF 0.5 GRAM N-METHYLCYCLOHEXENYLMETHYL BARBITURIC ACID-SODIUM SALT

The arrow indicates the time of administration of the agent.

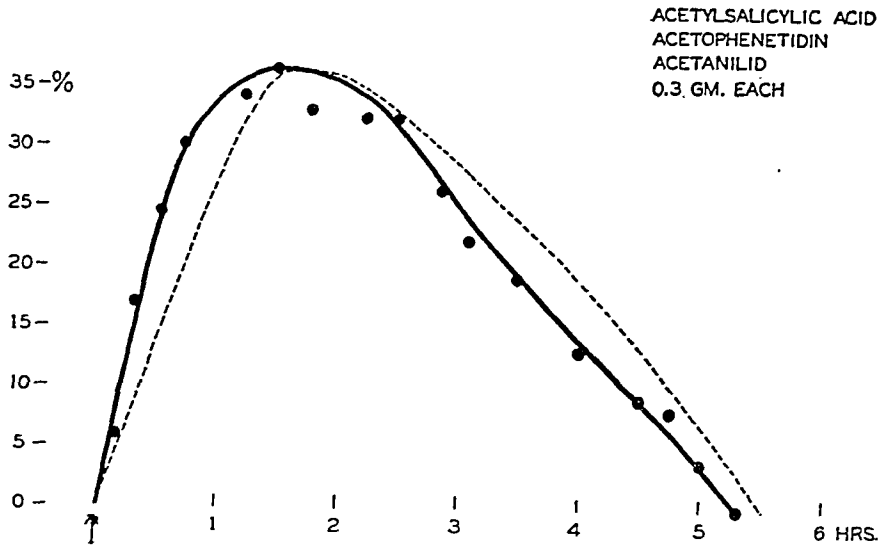


FIG. 16a. THE AVERAGE PAIN THRESHOLD ELEVATION FOR THREE SUBJECTS RESULTING FROM THE ADMINISTRATION OF THE COMBINATION OF ACETYSALICYLIC ACID, ACETOPHENETIDIN AND ACETANILID, 0.3 GRAM EACH (SOLID LINE)

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#### *Other combinations*

It is important in appraising the action of combinations to separate clearly the resultant pain threshold-raising action of a combination from other desirable effects. It is readily appreciated

that one may wish to combine an agent with good pain threshold-raising action with one having suitable sedative and hypnotic action, as well as other effects on mood and mentation.

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sedative effects were summative even though the threshold-raising effects were not.

The sodium salt of N-methylcyclohexenylmethyl barbituric acid ("Evipal") in 0.5 gram amounts was administered orally in combination with 0.3 gram of acetanilid. One experiment, representing 3 series of observations, was performed. No increase in the pain threshold-raising effect of acetanilid was observed. However, effects other than threshold-raising were important. Unsteadiness of gait, difficulty with skilled manual movements, difficulty in mentation, and barely controllable lethargy were noted. Diplopia was present. Again, the opinion shared by the three observers was that the sedative and hypnotic effects were summative.

A combination of a barbiturate and acetylsalicylic acid was also investigated (Figure 16b). The pain threshold-raising effect of 0.5 gram of N-methylcyclohexenylmethyl and 0.3 gram acetylsalicylic acid was ascertained in one experiment, representing 3 series of observations. The threshold-raising effect was no greater than that from 0.3 gram acetylsalicylic acid alone. The total action as expressed in hours per cent was not increased. However, other effects were

greatly increased over those of acetylsalicylic acid alone and even more evident than after the barbiturate alone. Diplopia, unsteadiness of gait, difficulty in mentation and profound lethargy were noteworthy.

*Comment.* It may be said, in general, of the combinations studied that the pain threshold-raising effect of a combination is no greater than that of its most effective ingredient, but that the sedative and hypnotic effects, as well as motility disturbances, may be summative. There may be valid grounds for giving agents such as codeine and barbiturates with such substances as acetylsalicylic acid, acetophenetidin and acetanilid, if one desires to achieve additive mood and mentation effects with antipyretic and stable threshold-raising effects.

#### SUMMARY AND CONCLUSIONS

1. Quantitative measurements of the pain threshold were made by irradiating 3.5 square centimeters of skin surface for 3 seconds. The intensity of radiation which barely evoked pain was denoted as the pain threshold. The threshold-raising action of various non-opiate analgesic

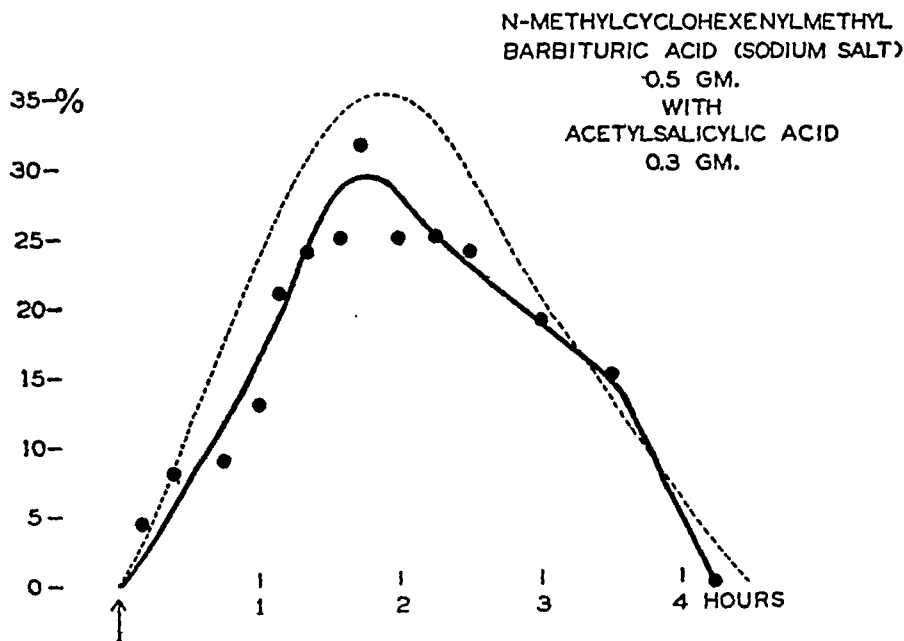


FIG. 16b. THE AVERAGE PAIN THRESHOLD ELEVATION FOR THREE SUBJECTS (SOLID LINE) RESULTING FROM THE ADMINISTRATION OF THE COMBINATION OF N-METHYLCYCLOHEXENYLMETHYL BARBITURIC ACID-SODIUM SALT (0.5 GRAM) WITH ACETYLSALICYLIC ACID (0.3 GRAM)

The dashed line represents the effect of 0.3 gram acetylsalicylic acid alone.

agents was then ascertained in terms of the normal threshold.

2. Acetylsalicylic acid (oral) in quantities from 0.03 gram to 1.8 grams was thus assayed. The minimum effective quantity of this agent is 0.06 gram (0.9 mgm. per kilo body weight). The "saturation" quantity or the smallest amount with which the highest threshold-raising effect was attained was 0.3 gram. The highest threshold-raising effect of which the agent was capable was approximately 35 per cent above the control threshold, and the maximum threshold-raising effect for quantities of acetylsalicylic acid, in amounts from 0.06 gram to 1.8 grams, was attained in approximately 50 to 100 minutes.

3. The time-action curves of threshold-raising effect for acetylsalicylic acid revealed that essential elimination increased at a constant rate with quantities up to 0.3 gram. However, with larger quantities of the agent, there was acceleration of the essential elimination rate so that duration of effect with 0.9 gram or 1.8 grams differed but slightly.

4. The pain threshold-raising action of 0.3 gram, respectively, of acetanilid, acetophenetidin and aminopyrine was measured. In these amounts these agents had time-action curves similar to that obtained with 0.3 gram of acetylsalicylic acid.

5. Acetylsalicylic acid induced mild relaxation and lethargy. Acetanilid and acetophenetidin in comparable amounts induced greater relaxation and lethargy and difficulties in mentation. The therapeutic effectiveness of the latter agents rests in good part on their effects.

6. The pain threshold-raising action of 30 and 60 cc. of ethyl alcohol (oral) was measured. In these amounts the alcohol had a maximum threshold-raising action of 40 per cent above the control level. The larger amount had a longer duration but no greater threshold-raising action.

7. The pain threshold-raising action, as well as other observable effects, of acetylsalicylic acid, acetanilid, acetophenetidin and ethyl alcohol was relatively slightly reduced by pain. A uniform pain stimulus was introduced just before or during the first 60 minutes after administration of the agents. Thus, the antagonism between pain and threshold-raising action noted in the case of the

opiates is less evident or absent in the case of the above-mentioned agents.

8. Some effects of alcohol were akin to those of the opiates. Of special significance in both were the emotional states referred to as freedom from anxiety and feelings of contentment and detachment. While in these states the subjects perceived pain but they were indifferent to it. Thus like the opiates but perhaps to a lesser degree, alcohol accentuates the ability to dissociate pain perception from the pattern of reaction to pain.

9. The pain threshold-raising action of 1 cc. of trichlorethylene (inhaled) was measured. It had a maximum threshold-raising action of approximately 40 per cent above the control level.

10. The pain threshold-raising action of 0.5 gram of the sodium salt of N-methylcyclohexenylmethyl barbituric acid ("Evipal" brand) was measured. It had a maximum threshold-raising action of approximately 20 per cent above the control level and induced profound lethargy and defects in mentation.

11. Caffeine sodiobenzoate, ergotamine tartrate and quinine sulphate had no pain threshold-raising properties.

12. Various combinations of acetylsalicylic acid, codeine, acetanilid, acetophenetidin, the barbiturates and caffeine were studied. The pain threshold-raising effect of these combinations was no greater than that of their most effective ingredient. Sedative and hypnotic effects, as well as defects in motility, seemed to be summative. A combination of these agents is therapeutically valid if it aims to attain useful psychological effects coupled with pain threshold-raising action. It is not valid, however, if it be assumed that the pain threshold-raising effects of the different ingredients will summate.

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# THROMBOCYTOPEN: A CONFIRMATORY REPORT

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Ever since the announcement by Troland and Lee (1) in 1938 of a platelet-reducing substance in the spleens of patients with idiopathic thrombocytopenic purpura, numerous efforts have been made to confirm their findings. Troland and Lee (2) were able to repeat their work at a later date with the same results. Their charts depicting the fall in platelets following the intravenous injection of acetone extracts of spleens from patients with thrombocytopenic purpura into rabbits, a cat, a dog, and a monkey, and the negligible diminution in platelets following injection of a similar extract of normal spleens are indeed impressive. Confirming this initial work, we have been able to find only one article, that of Hobson and Witts (3). Here the decreases in numbers of the circulating platelets were not so great as those of Troland and Lee, but still great enough to seem conclusive.

Far more numerous are those who have failed to demonstrate the presence of thrombocytopen, using a similar experimental technique. Among these are Pohle and Meyer (4), Major and Weber (5), Hodge and Strong (6), Tocantins (7), and Moore (8). Torrioli and Puddu (9) showed that extracts (not acetone) of spleens from patients with thrombocytopenic purpura applied in highly concentrated doses injure the megakaryocytes in cultures *in vitro* of the bone marrow of guinea pigs. They also found that similar extracts of normal spleens possessed like properties, but to a lesser degree. Torrioli and Pusic (10) showed that aqueous extracts of normal spleens, when given to rabbits in large intravenous doses, caused a reduction in circulating platelets. Torrioli and Puddu (9) point out that Troland and Lee in their work used spleens from patients with blood dyscrasias, congenital hemolytic jaundice and Banti's disease. Troland and Lee also used splenic tissue from a patient who died of cardiac failure and from one whose spleen was removed because of a stab wound.

Our interest in this controversial subject was stimulated by the opportunity of obtaining at op-

eration the spleen from a patient whom we had followed for over a year and whose history and findings are summarized below. The other patient with thrombocytopenic purpura died before this work was undertaken, but fortunately the spleen, which had been obtained at autopsy, had been ground up and placed in acetone for possible future examination. The spleens used as controls were taken at autopsy from a patient who died of multiple myeloma and from one who died of moniliasis of the lungs with pulmonary hemorrhage. Platelet counts were done on both of these patients before death and were found to be normal.

Following is a summary of the cases used in this paper:

*Case 1, M. M.*, was a 22-year-old white American college student who was first seen on August 17th, 1938, complaining of bruising easily for the past 8 years. Her family and past histories were not contributory. Her present illness consisted of easy bruising, sometimes without known trauma, severe nose-bleeds, profuse menstrual flow, occasional oozing gums, and profuse bleeding from small cuts over this 8-year period. There was no history of the use of any drugs during this time. There had been no previous treatment prior to entry here except for a diet high in Vitamin C, which gave no relief.

On physical examination, temperature was 36.6° C., pulse 96, respirations 20, and blood pressure 112/58. She was a well developed and well nourished young woman in no distress. Color was good. Several petechiae were present on the feet. There were numerous ecchymotic areas under the tongue and on her body, the largest ones being on her left leg, warm, tender, with raised indurated central portions. The spleen and liver were not felt at the initial examination, but later were palpable. Examination was otherwise not remarkable.

Laboratory work included the following findings: RBC 5,180,000 per cmm., Hgb. 85% (S), WBC 6,720 per cmm., with 67% polymorphonuclear neutrophils, 30% lymphocytes, and 3% eosinophiles; platelets varied between 78,000 per cmm. and "2000 red cells were counted and no platelets found." Ivy bleeding time varied between 13 minutes and over 49 minutes. Clotting time (Lee-White) varied between 5 and 7 minutes. Clot retraction was incomplete at 24 hours. Urine, stool, and blood Wassermann were all negative.

For various reasons splenectomy was not done until



October 10th, 1939. The operation was uneventful and by the 6th post-operative day her platelet count had reached a high point of 1,996,800 per cmm., falling gradually thereafter to around a 500,000 per cmm. level. No further bleeding occurred.

Sections of the spleen, which weighed 116 grams, showed the sinuses to be irregularly dilated and the tissue to be infiltrated with well preserved red blood cells. The lymphoid follicles appeared normal, most of them having germinal centers. Around many of them were rings of extravasated red blood cells. The tissue contained considerable brown pigment. Sections of a small accessory spleen showed similar structure.

One hundred grams of ground spleen were used for preparation of the extract to be injected into rabbits.

*Case 2, S. N.*, was a 65-year-old Japanese farmer complaining of bleeding gums for 3 weeks. The family and past histories were not contributory. He had always been well up to the onset of the present illness which started 3 weeks before entry with bleeding from the gums followed by purpuric spots and ecchymoses all over the body, especially over the extremities. There was also oozing from the nose, and on one occasion bleeding for a few minutes from the left ear. Except for slight weakness he felt quite well. He denied taking any drugs prior to the onset of his illness and had had no subsequent treatment except a Vitamin C preparation and yeast.

On physical examination, temperature was 37° C., pulse was 88, respirations 22, and blood pressure 110/74. He looked well. There were fresh and regressing petechiae and ecchymoses all over the skin surface, especially the extremities. The gums were spongy and oozing blood. The cheeks, lips, and tongue were also oozing blood. The edge of the spleen was palpable 3 to 4 cm. below the left costal border. The liver was not felt. Examination was otherwise not remarkable.

The laboratory work included the following findings: RBC 4,450,000 per cmm., Hgb. 80% (S), WBC 9,500 per cmm. with 67% polymorphonuclear neutrophils, and 30% lymphocytes; platelets varied between 80,750 per cmm. and 14,000 per cmm., reticulocytes were 0.6% of erythrocytes. Bleeding time was 10 minutes. Clot retraction was extremely poor at 24 hours. Urine and blood Wassermann were negative. Stool was strongly positive for occult blood (Guaiac).

This patient's course in the hospital was steadily downhill, and in spite of repeated transfusions he died following a large hematemeses 9 days after admission.

After autopsy the anatomic diagnosis was as follows: "Purpura with hemorrhage, stomach, fatal. Pneumonia, bronchial. Pericarditis, subacute, mild. Tuberculosis, lungs, apical, healed with pleuritis, chronic, adhesive. Hyperplasia of prostate, nodular, with hypertrophy of bladder. Arteriosclerosis, general, mild. Gastritis, subacute." The spleen weighed 60 grams. The capsule was markedly wrinkled and of a slate gray color. On cut section, it was softer than normal and the markings were moderately prominent. On microscopic examination, the malpighian bodies were poorly outlined. The trabeculae

and capsule were moderately thickened. The pulp was almost devoid of red blood cells. It was highly cellular. Many of the cells were large and could be identified as reticulum cells. Others were morphologically myeloid cells. One section showed a considerable deposit of golden brown granular pigment.

Marrow from a rib, the sternum, and a vertebral body was moderately hyperplastic, with abundant elements of both the erythrocytic and myelocytic series. Megakaryocytes were slightly increased in counts from smears. Differential count of hematopoietic cells was as follows: Primitive blasts 1%, myelocytes and metamyelocytes 76.5%, polymorphonuclear neutrophils 2%, eosinophils 1.5%, endothelial cells 1%, plasma cells 1.5%, normoblasts 16%, megakaryocytes 0.5%.

Sixty grams of ground spleen were used for preparation of the extract to be injected into rabbits.

*Case 3 (Control), L. L.*, was a 16-year-old Chinese schoolgirl who had been followed in this hospital for 11 years because of recurrent bouts of bronchopneumonia and hypochromic microcytic anemia. Guinea pigs inoculated with the patient's sputum showed lesions from which a *Monilia* was isolated. Her last entry on January 6th, 1940, was because of hemoptysis for 24 hours before entry.

On physical examination the temperature was 40° C., the pulse 140, the respirations 64, and the blood pressure 118/45. She was acutely ill, cyanotic, semi-stuporous, and bringing up moderate amounts of bright red blood. Other than this, positive findings were confined to the chest, both lungs being full of coarse râles anteriorly and posteriorly. The liver and spleen were not felt.

Laboratory work was as follows: RBC 4,400,000 per cmm., Hgb. 64% (S), WBC 27,200 per cmm. with 90% polymorphonuclear neutrophils, and 8% lymphocytes; platelets were 312,000 per cmm. No urine was obtained. Stool was negative for occult blood.

Death occurred 3 hours after entry.

The autopsy diagnosis was: Hemorrhage, lung recent, with (a) Hemorrhage, lung old; (b) Siderosis, lung, marked; (c) Fibrosis, lung, marked; (d) Carnification, lung; (e) Pneumonia, bronchial, mild; (f) Fibrosis, lymph node, peribronchial; (g) Siderosis, lymph node, peribronchial; (h) Emphysema, lung. Arteriosclerosis, generalized, mild. Cyst, ovary, unilocular.

The spleen weighed 175 grams. The surface was smooth and the capsule finely wrinkled. On section the pulp was soft but not mushy and could not be scraped off with a knife. The tissue was everywhere dark red save for multiple and well defined malpighian bodies measuring between 1 to 2 mm. in diameter. On microscopic examination, the capsule and trabeculae were thin. There was no fibrosis of parenchymal tissue. The malpighian bodies were large. A few of the sinuses were dilated and engorged with blood; most, however, were collapsed and there were large lakes of coagulated edema fluid as well as much recent hemorrhage in the intersinusoidal spaces of the pulp. Many small brown pigment granules

were scattered about. Polymorphonuclear neutrophils were not frequent.

Bone marrow from the sternum showed about 20 to 30 per cent of the marrow substances to be replaced by fat. Normoblasts and clusters of erythrocytes were common. Myelocytes and megakaryocytes were abundant.

One hundred grams of ground spleen were used for preparation of the extract to be injected into rabbits.

*Case 4 (Control), J. McA.*, was a 73-year-old white farmer who complained of a lump on the breast bone for 16 months and a sore right shoulder for 6 months.

On physical examination, the essential findings were a blood pressure of 186/104, moderate arteriosclerotic changes in the retinal and peripheral vessels, a tender, firm swelling at the upper end of the right humerus, and a firm rubbery mass over and attached to the upper sternum.

Laboratory work was as follows: RBC 4,000,000 per cmm., Hgb. 80% (S), WBC 9000 per cmm. with 75% polymorphonuclear neutrophils and 20% lymphocytes; platelets were 346,000 per cmm. Urine showed Bence-Jones protein. Stool showed no occult blood. Bone survey showed multiple areas of bone destruction.

The patient's course was steadily downhill. Autopsy diagnosis was multiple myeloma, plasma cell type—sternum, clavicle, skull, humerus, ribs, vertebrae, ilia.

The spleen weighed 125 grams and had a smooth, slightly wrinkled capsule. On section, the tissue was soft, dark reddish purple, with the pulp scraping away fairly readily with the knife edge. On microscopic examination, there were large areas of red blood cell extravasation. The follicles were small and without germinal centers.

Sections of the bone marrow free of tumor were normal save for a rather high number of normoblasts.

One hundred grams of ground spleen were used for preparation of the extract to be injected into rabbits.

#### METHODS

In preparing the extracts from these spleens the same procedure was used throughout, with one exception noted below. The spleens were removed direct from the operating room or morgue and immediately ground by running once through a kitchen meat grinder. The grindings were weighed and placed in five volumes of reagent acetone. The mixture was then placed in the ice-box, was frequently shaken, and was allowed to remain there for a minimum of 3 weeks. The acetone from the spleen of Case 1 was distilled off by heat in a water bath. Evaporation was used with the remaining three. The resulting brownish, sticky residue was then shaken thoroughly with 100 cc. of distilled water and the resulting suspension filtered first through coarse filter paper and then through a Seitz filter into a sterile suction flask.

Male rabbits weighing between 2 and 2.5 kilos were used for injection, a new rabbit being used for each injection. Prior to injection platelet counts were done at varying intervals for 3 to 5 days. This was done both

for purposes of standardization and also to ascertain whether there was any marked difference in the count at different times of day or night. We found none. The direct method of Rees and Ecker was used, and each count was checked in a separate counting-chamber. Both chambers were thoroughly searched for clumping, and if any was found the count was repeated. Pipettes were shaken in a mechanical shaker for 10 minutes and the platelets allowed to settle in the counting-chamber for another 10 minutes before the count was started. The diluting solution was passed through a Seitz filter every 24 hours to exclude the possibility of any dust particles or bacteria confusing the count.

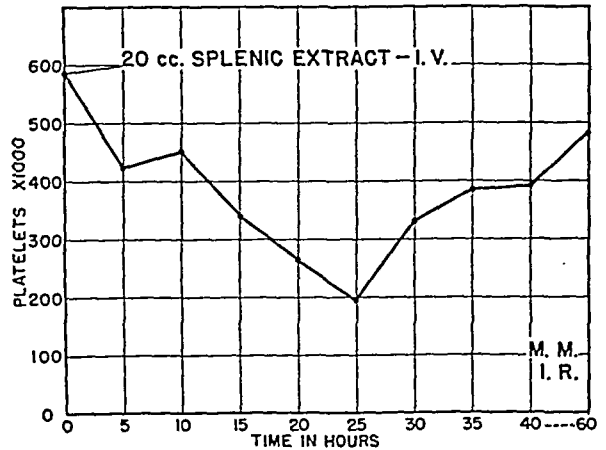


FIG. 1. INJECTION OF FIRST SAMPLE OF SPLENIC EXTRACT OF PATIENT M. M. WHO HAD IDIOPATHIC THROMBOCYTOPENIC PURPURA

First rabbit, injected by R.

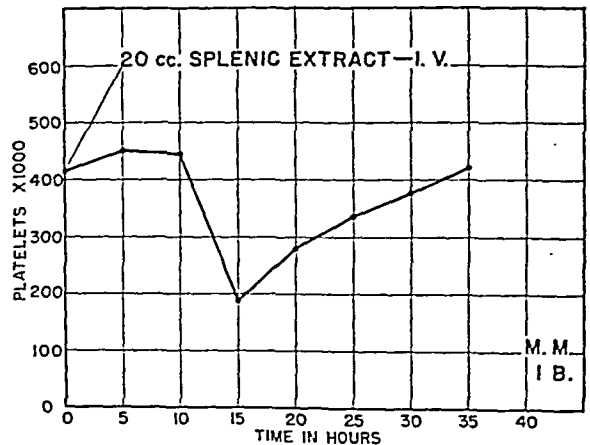


FIG. 2. INJECTION OF SECOND SAMPLE OF SPLENIC EXTRACT OF PATIENT M. M.

Second rabbit, injected by B.

Twenty cubic centimeters of the aqueous solution prepared as stated above were injected into the marginal ear vein of a rabbit after a final control count had been done. These control counts and the counts done at intervals of 5 hours thereafter are recorded in the accompanying charts.

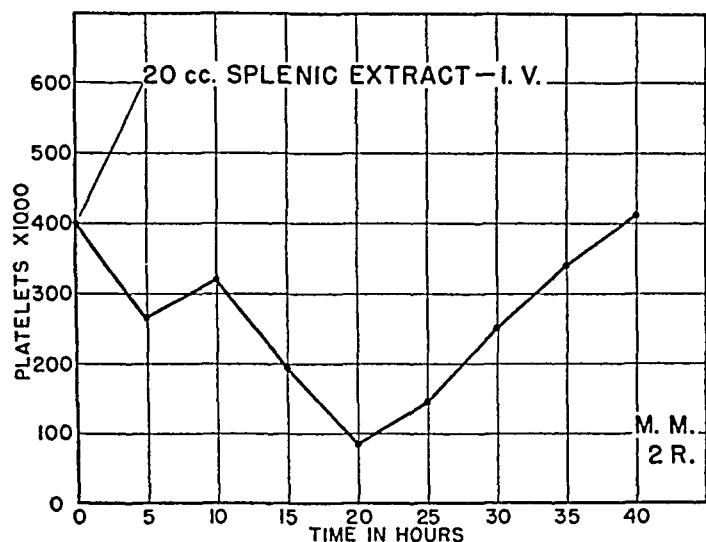


FIG. 3. INJECTION OF THIRD SAMPLE OF SPLENIC EXTRACT OF PATIENT M. M.

Third rabbit, injected by R.

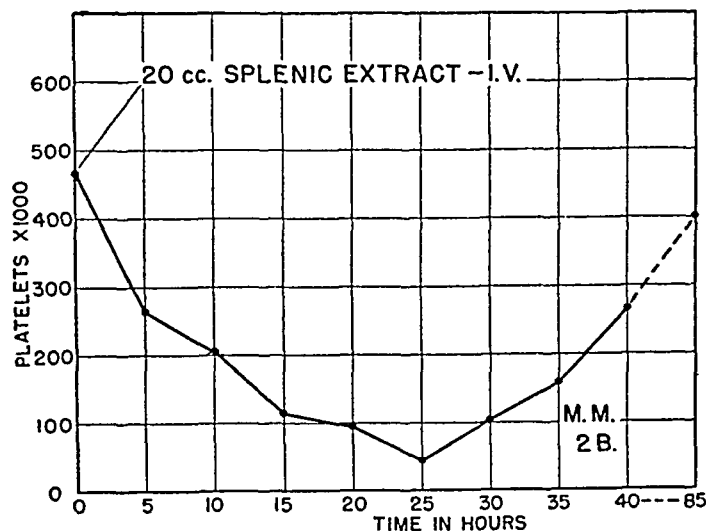


FIG. 4. INJECTION OF FOURTH SAMPLE OF SPLENIC EXTRACT OF PATIENT M. M.

Fourth rabbit, injected by B.

#### RESULTS

In all the rabbits injected with extract from spleens of thrombopenic purpura patients there was a distinct drop in platelets. Many control counts before injection indicate that these drops are significant. Extracts from control cases uniformly failed to produce any definite fall in the platelet count.

We have almost nothing to add to the comments already set forth in preceding papers. We have carefully studied the published case reports and can find nothing to explain why the platelet reducing substance is sometimes found and more often not. Almost all of the reports concern cases which were undoubtedly instances of idiopathic thrombocytopenic purpura. None of the abstracts of the pathological findings gives any clues as to this discrepancy. The technique used by the different workers was too similar to permit any explanation along this line. Why we failed to obtain any marked drop in the platelet level at 5 hours, as did Troland and Lee, but for the most part

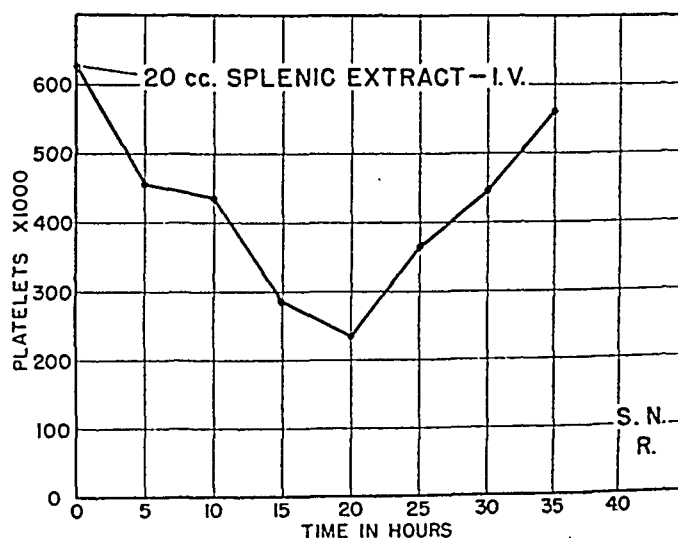


FIG. 5. INJECTION OF FIRST SAMPLE OF SPLENIC EXTRACT OF PATIENT S. N., WHO HAD IDIOPATHIC THROMBOCYTOPENIC PURPURA

Fifth rabbit injected by R.

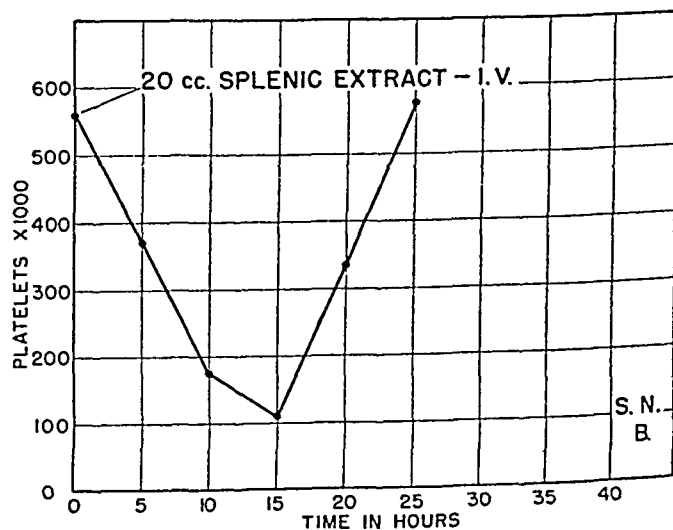


FIG. 6. INJECTION OF SECOND SAMPLE OF SPLENIC EXTRACT OF PATIENT S. N.

Sixth rabbit injected by B.

obtained slower falls and rises, adds still another unanswered question.

### SUMMARY

1. Spleens from two patients with idiopathic thrombocytopenic purpura and from two control patients with no evidence of this disease were extracted according to the method of Troland and Lee.

2. The splenic extracts were injected into healthy young male rabbits, the platelets of which

had been counted and repeatedly checked beforehand.

3. In the rabbits injected with extracts from the spleens of the patients with idiopathic thrombocytopenic purpura, the platelets dropped markedly in number, whereas in rabbits injected with control extracts there was no appreciable drop.

4. We consider these data as confirmatory of the work of Troland and Lee.

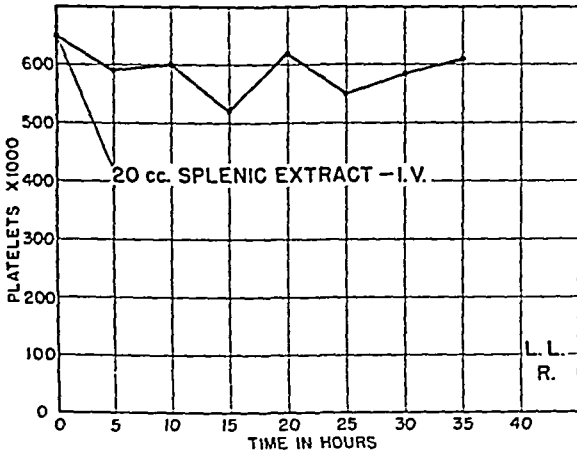


FIG. 7. INJECTION OF FIRST SAMPLE OF SPLENIC EXTRACT OF PATIENT L. L., WHO DIED OF MONILIASIS AND HAD NO EVIDENCE OF IDIOPATHIC THROMBOCYTOPENIC PURPURA

Seventh rabbit, injected by R.

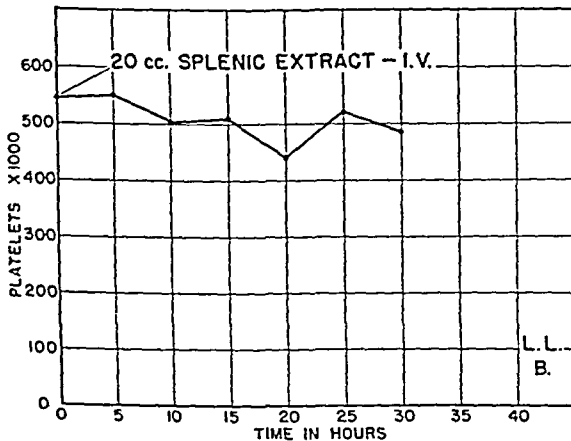


FIG. 8. INJECTION OF SECOND SAMPLE OF SPLENIC EXTRACT OF PATIENT L. L.

Eighth rabbit, injected by B.

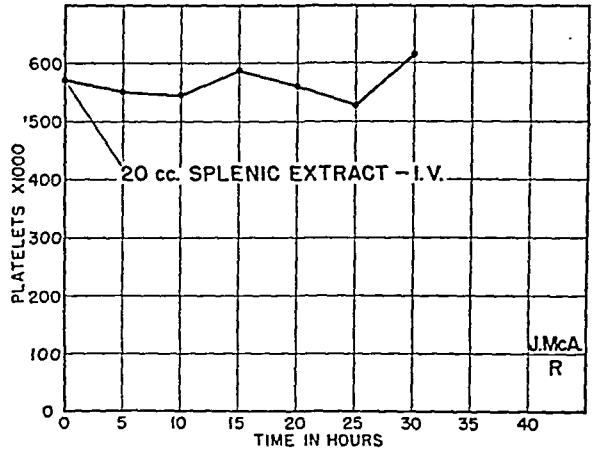


FIG. 9. INJECTION OF FIRST SAMPLE OF SPLENIC EXTRACT OF PATIENT J. MCA., WHO DIED OF MULTIPLE MYELOMA AND HAD NO EVIDENCE OF IDIOPATHIC THROMBOCYTOPENIC PURPURA

Ninth rabbit, injected by R.

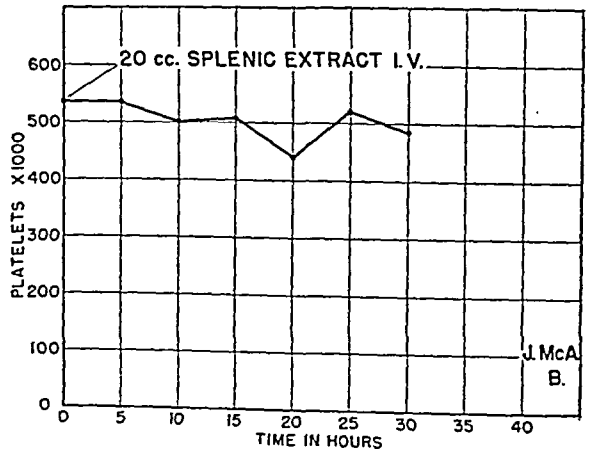


FIG. 10. INJECTION OF A SECOND SAMPLE OF SPLENIC EXTRACT OF PATIENT J. MCA.

Tenth rabbit, injected by B.

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# THE MEASUREMENT OF CARDIAC OUTPUT. AN IMPROVEMENT OF THE ACETYLENE METHOD PROVIDING AN INHERENT CHECK

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The method of Marshall and Grollman (1) for measuring the arteriovenous oxygen difference by forcible rebreathing of a mixture of air, oxygen and acetylene has been criticized on the grounds that gas samples are taken during a period when significant amounts of blood containing acetylene are returning to the lungs; that is, from 15 to 23 seconds after the beginning of rebreathing. On the other hand, if samples are taken too soon after the beginning of rebreathing they may contain bag or lung air which has not been completely mixed. The three sample technique (2) which provides for the calculation of duplicate results for arteriovenous difference was introduced to overcome these objections. However, the use of this procedure in 76 experiments in our laboratory yielded second values which averaged 12 per cent higher than the first values, indicating increasing recirculation of acetylene. Hence it is not justifiable to accept even the first value, because without clear-cut checks between the two figures either or both of them may be subject to errors due either to recirculation or to incomplete lung-bag equilibrium. An attempt was made to evaluate these two sources of error and to devise a method which eliminated them.

## EXPERIMENTAL

**Multiple expiratory samples.** The valve as described by Grollman was modified to permit withdrawal of six samples during successive expirations at the time of forcible rebreathing of the acetylene air mixture. The arteriovenous oxygen difference calculated from successive pairs showed a progressive rise when the samples were taken from 12 to 30 seconds after the beginning of rebreathing. During the first 10 seconds the results were irregular, often very low or very high, and successive values frequently varied widely.

**Multiple samples of "alveolar" and "bag" gas.** The valve was modified further by the introduc-

tion of a quickly acting flap valve between the bag and the sampling tube connections at the mouthpiece and provision was made for sampling at the

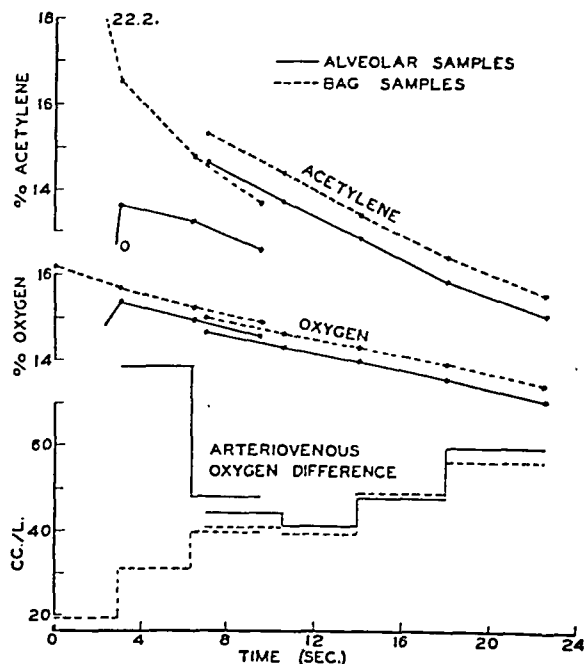


FIG. 1. ACETYLENE AND OXYGEN CONCENTRATIONS IN ALVEOLAR AND BAG GASES DURING TWO REBREATHINGS, TOGETHER WITH THE CORRESPONDING ARTERIOVENOUS OXYGEN DIFFERENCES

Samples were taken at the end of successive expirations. The first experiment extended through the first three breaths (9.5 seconds). In the second experiment sampling was started following the third breath (7 seconds) and carried on for five successive breaths.

other end of the bag as well. At the end of successive expirations the flap valve was closed momentarily and samples composed principally of alveolar gas were taken at the mouthpiece; other samples, representing a mixture of some alveolar gas with all of the gas from the dead spaces in the bag, valve, and upper respiratory tract, were taken simultaneously from the end of the bag.

## ARTERIOVENOUS OXYGEN DIFFERENCE FROM SUCCESSIVE ALVEOLAR AND BAG SAMPLES.

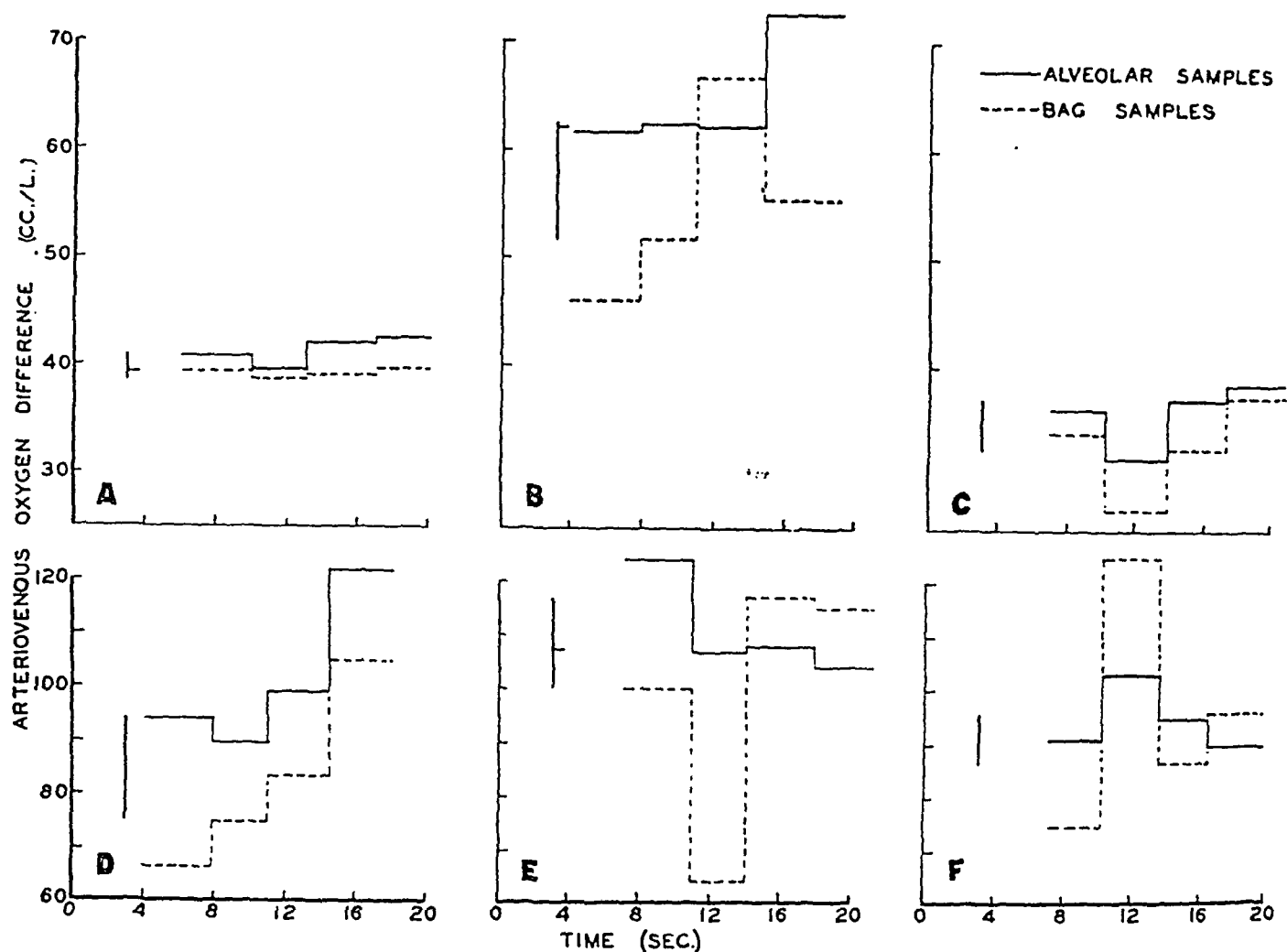


FIG. 2. GRAPHS OF ACTUAL RESULTS OF REBREATHING TO ILLUSTRATE TYPES OF ACCURATE AND INACCURATE DETERMINATIONS

The length of the vertical bar at the left of each figure is the range within which the value for arteriovenous oxygen difference can be assumed to lie. When a probable correct figure can be pointed out within this range, it is indicated by a horizontal line drawn from the vertical bar.

After lung-bag equilibrium had occurred the gas concentrations in successive "alveolar" and "bag" samples fell on approximately parallel lines. This situation is present early in the case of oxygen, since the difference between normal alveolar oxygen concentration and bag oxygen concentration at the beginning of rebreathing is relatively small. In a typical experiment (Figure 1) oxygen equilibrium was established in one breath. Some delay occurred in the case of acetylene, since initially the bag concentration was over 20 per cent and the alveolar concentration was zero. Acetylene equilibrium was established at the fourth breath (Figure 1). In the second rebreathing (Figure 1) sampling was begun after the third breath and both oxygen and acetylene

values were found parallel. After the establishment of equilibrium the decrease in acetylene values (acetylene difference) from breath to breath was the same in "alveolar" and "bag" gas; prior to this the decrease was smaller in the former than in the latter.

#### THE MODIFIED METHOD

The above-described experiments proving the difficulty in determining when lung-bag equilibrium has been reached with the Grollman technique and the demonstration that this difficulty is obviated by means of the above-described modification of the valve and method, led to the formulation of the present technique for measuring cardiac output by means of the forcible rebreathing of mixtures of air, oxygen, and acetylene.

The gas mixture is made up as described by Marshall

and Grollman (1) equal in volume to two-thirds to three-fourths of the vital capacity of the subject. Strenuous preliminary expiration leaving only the residual air in the lungs, together with a large amount of bag gas, favors rapid equilibration. The rebreathing is performed with the subject in bed elevated by a back rest to about 45°. Sampling is accomplished from both the mouthpiece and the bag, as described above, using the modified valve. Analyses are performed by means of a modified Haldane apparatus and the oxygen consumption is determined in duplicate by means of the Benedict-Roth apparatus.

Plotting the obtained oxygen and acetylene values (Figure 1) does not permit accurate judgments regarding the slope of lines in various periods, since estimation of the time of sampling is subject to an error of at least half a second. On the other hand, values for arteriovenous oxygen difference calculated from these data are suitable for detecting small errors, since slight inaccuracies in timing samples are not important in this connection. A definite but short plateau in the values for arteriovenous oxygen difference is evident before recirculation occurs, in this case some time between 14 and 18 seconds after the beginning of rebreathing. This plateau represents the true value for arteriovenous oxygen difference uninfluenced by errors due to lack of lung-bag equilibrium or recirculation of acetylene.

The time of this plateau is variable from person to person and in the same subject from day to day. Figure 2A represents a continuous plateau with equilibration after 6 seconds and little or no recirculation at 20 seconds. This result is no more accurate than the short but definite plateau in Figure 1. Figure 2D illustrates the occurrence of recirculation without establishment of lung-bag equilibrium. If six respirations are made and samples of "alveolar" and "bag" gas are taken after the second and each succeeding expiration, and the rebreathing is completed in about 20 seconds, a plateau with values calculated from "alveolar" and "bag" samples coinciding is usually demonstrated. This ordinarily occurs between the third and fifth expirations and, when this is so, analyses of the second, third, and fourth samples from each source are sufficient. It is desirable to have the other samples available, however, because when irregular results are encountered in the calculations from these six samples a plateau may be demonstrated earlier or later from the other two pairs of samples. If not, the cause of the unsatisfactory result may often be identified and the next rebreathing modified by changing the sampling time or the volume of gas in the bag.

The determination can be accepted as accurate only when the values calculated from "bag" samples coincide with those calculated from "alveolar" samples, indicating lung-bag equilibrium during a plateau consisting of successive values in agreement, thus signifying the absence of the errors to be described below.

The principles used in evaluating each result are also illustrated in Figure 2. Some of the individual values in each graph have been disregarded as obviously due to errors described below. The range within which the

arteriovenous oxygen difference can be said to lie, after the obvious errors have been discarded, is indicated in each graph by a vertical line at the left. When the range is small it is valid to accept the midpoint of that range as the result, subject to an error of half that range. In instances in which the range is larger it is safer to consider the range within which the value may possibly fall; acceptance of the most probable value within that range, as in Figure 2B, should wait upon additional determinations.

*Sources of error.* (1) Leaks. Very strenuous breathing, either inspiration or expiration, increases the incidence of mouthpiece and nose-clip leaks. Leaks during sampling may also occur. Irregularities in results caused by these factors are readily detected (Figure 2B and C).

(2) Small lung-bag volume. Small total lung-bag volume, due either to small initial volume or to withdrawal of frequent or large samples, causes an error resembling that due to recirculation. This error increases progressively from interval to interval as the lung-bag volume is reduced, since the samples show progressively larger oxygen and acetylene percentages in relation to true alveolar gas, and since more acetylene than oxygen usually is absorbed. In practice, it is not always possible to distinguish between the effect of recirculation and this error.

(3) Inadequate mixing. Errors due to incomplete mixing are revealed by marked differences between "bag" and "alveolar" gas concentrations. Calculations of arteriovenous oxygen difference from data obtained before lung-bag equilibrium is attained, yield falsely high values if "alveolar" gas is used and falsely low values if "bag" gas is used (Figure 1). Errors introduced by lack of equilibrium of acetylene and oxygen are in opposite directions; since the lack of acetylene equilibrium is more marked at the beginning and more persistent, the total errors are in the direction of the errors due to acetylene imbalance.

(4) Recirculation. Errors due to this factor are readily detected by the absence of a definite plateau. This error can occur as early as 15 seconds after the beginning of rebreathing but in some instances there is no evidence of recirculation up to 20 seconds. Individuals show considerable day-to-day variation in this respect. When samples are taken after each expiration this error can be identified more accurately than with few samples. Samples from two sources help to distinguish errors due to recirculation from other errors.

(5) Analytical errors. Analytical inaccuracies introduce larger errors in the final result when sampling is frequent and the differences between successive samples are small; but if duplicate values agree within 0.03 per cent, the final error is seldom large enough to be important. Nevertheless, the accuracy of gas analyses is a factor which largely determines the accuracy of this method.

*Accuracy of method.* Eighty-two rebreathings have been performed on five subjects, using the method as de-



## CHANGE IN ARTERIOVENOUS OXYGEN DIFFERENCE.

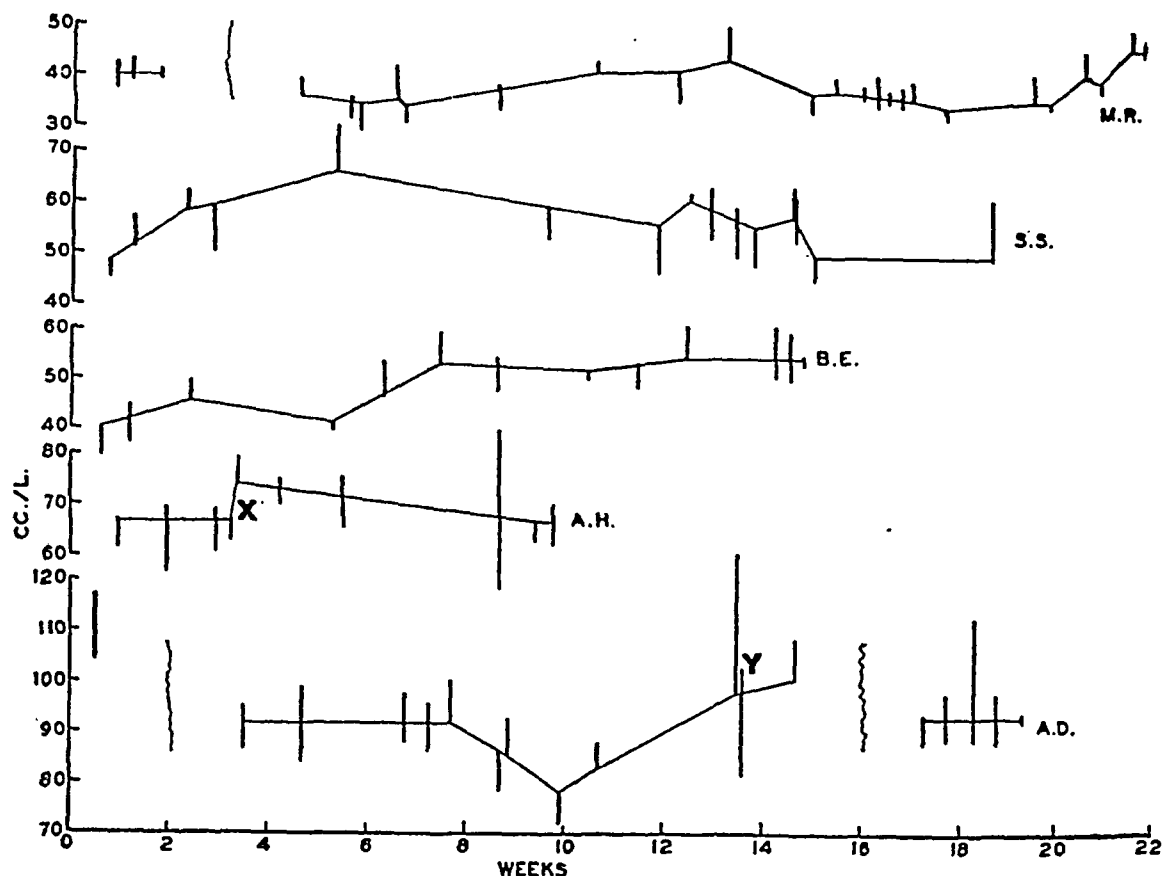


FIG. 3. THE VERTICAL BARS REPRESENT THE RANGE OF ARTERIOVENOUS OXYGEN DIFFERENCE DETERMINED FOR EACH REBREATHING AS IN FIGURE 2

The values are those included in Table I. The abscissa represents the time relationship of the determinations. The bars representing the determinations on each individual are connected by a line drawn as nearly horizontal as possible through the whole series. This indicates the minimum variation from time to time.

TABLE I

Range of accuracy of arteriovenous oxygen difference in 82 determinations on two normal subjects and three patients \*

Range (Per cent of midpoint)	Number of determinations					Total sub- jects
	Subjects					
	Normals		Patients *			
	S. S.	B. E.	M. R.	A. D.	A. H.	
0-5.....	1	3	4	1		9
6-10.....	3	2	11	8	5	29
11-15.....	5	4	8	5	2	24
16-20.....	5	4	3	1	2	15
21+.....	1			3	1	5

\* See text for diagnosis.

Subjects were sitting in bed with the back rest elevated to an angle of 45°.

scribed above. Subjects S. S. and B. E. were normal, subjects A. H. and A. D. had chronic rheumatic heart disease with symptoms of cardiac insufficiency but no

râles in the lungs, and subject M. R. was convalescing from rheumatic fever with intermittent slight fever but no increase in sedimentation rate or leukocytosis; she had been in bed for over a year at the time of study. All determinations were made in the morning under basal conditions.

Maximum and minimum values for each rebreathing are indicated in Table I and Figure 3, individual values obviously in error being discarded according to principles illustrated in Figure 2. No completed rebreathings are omitted, however. In Table I the difference between maximum and minimum possible values is expressed in percentage of the midpoint between them so that the greatest possible error of this midpoint is half the difference between the maximum and minimum values. In 38 of the 82 determinations the error was 5 per cent or less and in 62 of the 82 it was 7.5 per cent or less.

The accuracy with which physiologic variation can be determined is limited by the precision of individual values. The minimum real change in these subjects is represented graphically in Figure 3. The actual variation may exceed this. For example, the pair of determinations made on successive days on patient A. H. (marked X, Figure

3) shows a definite minimum increase of 6.5 cc. per liter in arteriovenous oxygen difference represented by the line connecting the bars, and a maximum possible real increase of 15.3 cc. per liter (the difference between opposite ends of the bars). This change could be expressed as  $10.9 \pm 4.4$  cc. per liter.

At times 2 determinations, each of which is unsatisfactory, may provide reasonably good evidence that the arteriovenous oxygen difference lies within a narrow range as shown in the two observations marked Y (Figure 3, patient A. D.).

#### DISCUSSION

The studies described here demonstrate that complete mixing in the lung-bag system used may occur in two breaths and is usually achieved by four breaths. This is earlier than was indicated by the experiments of Grollman and Marshall (4) who concluded that equilibration required 15 seconds. Gladstone and Dack (5), however, have pointed out the fallacy in the work of Grollman and Marshall. The present studies confirm the observations of Gladstone and Dack in regard to the excretion of excessive amounts of  $\text{CO}_2$  during the first 15 seconds of rebreathing; lung-bag equilibrium could not become apparent in Grollman and Marshall's experiments (4) while an excess of  $\text{CO}_2$  was being removed from the blood by deep breathing. The contention of Grollman and Marshall that rebreathing must proceed for 15 seconds before lung-bag equilibration is accomplished is therefore apparently erroneous. The modified method described here makes possible the certain detection of inadequate lung-bag mixing and so avoids the errors pointed out by Grollman (1) and Hamilton, Spradlin, and Saam (3).

That recirculation of blood containing acetylene occurs in less than 20 seconds has been shown by Hamilton *et al.* (3), Starr and Collins (6) and Gladstone (7), as well as by the results of the experiments described here. However, the modified method suggested obviates errors due to this factor.

Although the modifications suggested here make the method more involved, it is less complicated for the subject than partial inspiration from each of two bags, as in the Gladstone modification (8). Reduction of the volume of the lung-bag system, as recommended by McMichael (9), may serve to increase rather than decrease the errors of the original Grollman method. Time expended in

making gas analyses is increased two- to fourfold by our method but this increase is made worthwhile by considerations outlined above.

While this method makes possible the recognition of errors due to incomplete lung-bag equilibrium, recirculation, leaks in the lung-bag system and sampling tubes and analytical inaccuracies, it should be pointed out that there are other uncertainties in the method. Some of these are: incomplete saturation of blood by acetylene due to circulatory shunts from one side of the heart to the other and to abnormalities of the lung; changes of alveolar oxygen concentration by deep breathing when alveolar oxygen falls too low during the determination or when the arterial oxygen saturation is below normal during normal respiration (the alveolar oxygen, and therefore the arterial saturation, are raised during rebreathing); changes in the blood which modify the solubility coefficient of acetylene; and diffusion of acetylene into tissues of the respiratory tract other than blood. These errors are probably small or of only occasional occurrence and have been discussed elsewhere.

#### CONCLUSIONS

1. In determining arteriovenous oxygen difference with the acetylene method, multiple samples of "alveolar" and "bag" gas make it possible to identify the establishment of lung-bag equilibrium of the acetylene-air mixture and the beginning of recirculation of acetylene-containing blood to the lungs in each instance. Analytical inaccuracies and leaks, as well as most of the many irregularities encountered in a procedure of this type, are identified.

2. The time required to achieve lung-bag equilibrium and the time of the first appearance of recirculation are variable, but they usually occur much earlier in the period of rebreathing than Grollman (2) indicated.

3. The interval between the establishment of equilibrium and the occurrence of recirculation varies, but is usually long enough to allow at least two complete respirations. This is sufficient for an accurate determination of arteriovenous oxygen difference.

4. Seventy-six per cent of 82 determinations were in error by less than 7.5 per cent.

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# THE LIVER LIPIDS IN NORMAL HUMAN LIVERS AND OF CIRRHOSIS AND FATTY INFILTRATION OF THE

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The distribution of lipids in the liver has been studied rather thoroughly during the past 10 years but most of the observations have been on the livers of normal or diabetic dogs (1, 2, 3) and on rats (4, 5). Reports of the fat content of human livers, either normal or pathological, have in comparison been limited (6, 7, 8). Such analyses on human livers are of increasing interest because the experimental studies on the production of fatty livers in animals have suggested not only new etiological factors but also means by which fatty infiltration of the liver might be prevented (9, 10). One factor obviously capable of influencing the deposition of fat in the liver is the external secretion of the pancreas. This has been shown by the fact that, when the pancreatic ducts were ligated in dogs, marked fatty infiltration of the liver occurred (11, 12). This fatty infiltration was not accompanied by any demonstrable disturbance of carbohydrate metabolism but was similar in degree and type to the fatty infiltration of the liver observed in the depancreatized dog. In both the dog with the ligated duct and the depancreatized animal choline in doses of 2 grams daily was capable of preventing the deposition of fat in the liver. Recently we observed another interesting fact (13), namely, that regardless of the pathological state of the dog, fatty infiltration of the liver did not occur if in place of whole meat the dogs were fed a diet containing the dried meat powder from which the water-soluble extractives had been removed.

In humans, fatty livers have been observed at postmortem in cases of chronic alcoholism, diabetes mellitus, marked obesity and occasionally in certain endocrine disorders. The great majority of such observations have been based on the gross and microscopic appearance of the livers. Chemical analysis of the liver gives not only a more exact idea of the total amount of lipid but it is the only means of determining the nature of the lipid changes that may have taken place.

TABLE I

*The rate of change in the analytical values of the lipids of a normal, intact dog liver kept in refrigerator*

Sample number	Time elapsed	Total lipids	Total fatty acids	Total cholesterol	Phospholipids	Neutral fat
	hours	per cent	per cent	mgm. per cent	per cent	per cent
1	0.5	3.41	2.36	239	2.30	0.87
2	4.5	3.45	2.43	241	2.19	1.02
3	23.0	3.19	2.29	231	1.82	1.13
4	46.0	3.36	2.45	245	1.80	1.32

## PROCEDURE

In this study we wished to reexamine the earlier observations of the lipid content and distribution in normal human livers and, in addition, to study the changes that might occur as a result of liver damage. The normal group consisted of 25 subjects killed in accidents in whom no gross pathological findings were observed at autopsy except those connected with the immediate cause of death. The pathological group consisted of 25 patients with well established histories and evidences of chronic alcoholism and 5 cases of cirrhosis of the liver with no history of alcoholism. Liver samples were obtained in most of the cases within 24 hours after death. In order to check the effect of any postmortem changes on the various lipid fractions, fresh specimens of dog liver were kept in the ice box and determinations were done at intervals of ½, 4½, 23 and 46 hours (Table I). The only significant change found was in the apparent concentration of phospholipids (calculated as lecithin) and of neutral fat, which is discussed later. Lipids and their fractions were done by the methods previously described from this laboratory (1).

## RESULTS

The normal group (Table II) consisted of 20 males and 5 females. The ages varied from 4 to 81 years. The average total liver lipid for the group was 4.98 grams per 100 grams of wet liver. The range was from 2.42 to 8.41 grams per cent. In 19 of the cases the total lipid did not exceed 5.41 grams per cent. In all probability in the normal liver the concentration of total lipid will not exceed 8 grams per cent. This corresponds with the values reported by Breusch and Scala-

TABLE II  
*Liver lipids in normal subjects*

Case number	Age	Sex	Liver weight	Total lipids	Fatty acids	Cholesterol		Phospholipids	Neutral fat
						Free	Total		
			grams	grams per cent	grams per cent	mgm. per cent	mgm. per cent	grams per cent	grams per cent
8	50	M	1500	3.26	2.34	197	247	2.42	1.35
12	30	M	1250	8.41	6.98	199		1.81	6.0
15	10	M	950	3.55	2.25		297	1.73	1.12
16	74	F	1200	6.35	4.30		296	2.53	2.72
17	35	M	1300	4.92	2.98		388	2.34	1.38
4	34	M	1760	4.25	2.69	217	249	3.03	0.69
35	45	M	1630	4.42	3.54		240	2.42	2.03
47	30	M	1820	2.42	1.62		256	1.47	0.63
49	55	M	1800	6.22	4.37		309	2.54	2.70
50	40	M	1670	5.41	2.38		261	2.57	0.69
69	59	F	1370	4.08	2.68			1.94	1.40
60	55	M	1780	8.50	7.22			1.46	6.5
61	18	F	1200	4.81	3.06			2.16	1.67
63	14	M	1100	3.55	2.16			1.53	1.17
71	62	M	1500	4.26	2.95			1.67	1.90
72	55	M	1620	6.49	5.05			1.68	4.1
76	4	M	530	5.03	3.38				
77	35	F	1170	4.44	2.88				
80	39	M	1500	4.65	3.18				
81	49	M	1450	5.01	3.37				
85	35	M	1640	3.51	2.54				
89	45	M	1470	3.44	2.49				
90	81	M	1410	5.29	3.62				
94	50	M	1540	8.02	6.89				
100	39	F	1300	4.10	2.68				
Average			1430	4.98	3.50	204	283	2.08	2.26

brino (7) and by Kennaway and Leathes (8). It is also the upper limit of the liver lipid in normal dogs (1). Total cholesterol determinations were done in 9 of these cases. The average was 283 mgm. per cent. In 2 cases the total was over 300 mgm. per cent. The greater portion of cholesterol in the liver is present normally as free cholesterol (1). Neutral fat and lecithin determinations were done in 16 of the cases. The lecithin averaged 2.08 grams per cent, with a range from 1.46 to 2.57 grams per cent. The average value for neutral fat was 2.26 grams per cent with a range from 0.69 to 6.54 grams per cent. In only 2 cases was the concentration over 6 grams per cent and in only 2 cases was the concentration as low as 0.69 gram per cent. As one would expect, the higher neutral fat values were found in the cases with total lipids at the upper level, *e.g.*, 8 grams per cent. The total liver lipid in a normal individual may reflect to some extent the nutritional state or it may be related to the ingestion of food, particularly fatty foods.

In the group of 25 patients with alcoholic his-

tories (Table III) the findings in the livers present certain striking changes. The total lipid was elevated above 9 grams per cent in 13 cases, the highest being 34.8 grams per cent. Of the 13 cases with elevated total lipid, one had meningitis, 2 had pneumonia, 3 had jaundice with ascites and early cirrhosis, one had periportal fibrosis and cirrhosis, one had coronary sclerosis and one had tuberculosis. The increase in total lipid in these 13 livers was due to an increase in the neutral fat fraction. This is the type of change that is always found in fatty livers and is also seen in the livers of depancreatized dogs or dogs with ligated pancreatic ducts (10, 11). In the remaining 12 cases the total liver lipid varied from 2 to 7.07 grams per cent. Of these 12 cases, in addition to alcoholism, 3 were cardinals, 2 died following an accident, 1 had a hypernephroma, 1 was a patient with hemochromatosis and diabetes who was admitted in ketosis and was kept on a low fat diet and insulin, 2 had portal cirrhosis and 3 cases were uncomplicated. The complications may have had some effect on the total amount of lipid in the liver, particularly when associated with malnutrition or low fat diets. Undoubtedly, in these cases the phase of fatty infiltration had preceded the cirrhotic stage. The total cholesterol, measured in 16 of the 25 livers, was above 300 mgm. per cent in 10 cases. In 8 cases this elevated cholesterol was in livers in which the total lipid was elevated. The elevation was due apparently to an increase in the esterified fraction, as the free cholesterol averaged 254 mgm. per cent. The increase of cholesterol in the fatty livers was not proportional to the increase in neutral fat, *e.g.*, in Case 39 the neutral fat was 32.5 grams per cent and the total cholesterol was 317 mgm. per cent, whereas in Case 1, the neutral fat was 11.2 grams per cent and the total cholesterol was 406 mgm. per cent. In depancreatized dogs the cholesterol infiltration into the liver tends to parallel the fatty infiltration (10). The fact that this was not observed in this group of human livers may reflect the difference in the cholesterol content of the diet. Note should be taken of the fact that the majority of these patients had some other complication besides alcoholism.

In Table IV are reported the lipid values in 5 cases of cirrhosis of the liver uncomplicated by

TABLE III  
*Liver lipids in alcoholic patients (25 cases)*

Case number	Age	Sex	Liver weight	Total lipids	Fatty acids	Cholesterol		Phospho-lipids	Neutral fat	Pathology of liver and other disease processes
						Free	Total			
			grams	grams per cent	grams per cent	mgm. per cent	mgm. per cent	grams per cent	grams per cent	
56	35	F	2450	11.5	10.2		337	1.99	9.3	Fatty liver; streptococcus meningitis.
99	35	M	1780	27.0	26.8		317			Fatty liver.
75	41	M	1910	16.1	14.9					Lobar pneumonia; fatty liver.
65	40	F	1750	31.3	28.1			1.33	28.5	Fatty liver.
96	45	M	1450	3.36	2.05					Liver normal pathologically.
82	45	M	1720	15.3	14.1		265			Pulmonary tuberculosis; fatty liver.
66	50	M	1410	4.18	2.94			2.05	1.66	Cardiac failure. Liver normal.
91	50	M	1050	4.96	3.42					Slightly fatty liver.
84	38	M	1850	13.3	11.9					Pulmonary tuberculosis.
1		M	2350	12.7	11.0	273	406	1.30	10.5	Enlarged and fatty liver.
25	68	F	1450	4.91	3.55		287	1.58	2.66	C.P.C. of liver; coronary thrombosis; ascites.
28	50	M	1800	7.1	5.3		273	1.92	4.2	Subdural hematoma.
43		F	1500	5.29	2.87		267	2.70	1.12	
6	53	M	1500	10.0	8.1	219	321	2.20	6.9	Coronary sclerosis.
101	51	M	1650	3.83						Fractured skull; cirrhosis of liver.
102	47	M	1750	2.50						Enlarged heart; C.P.C. of liver.
103	55	M	2550	2.45						Hypernephroma; no metastases to liver.
58	50	M	1950	28.1	25.5		389	1.97	25.3	Pneumonia.
110	47	M	4000	15.4	14.0	257	360	1.20	13.8	Fatty liver with perilobular fibrosis; ascites; jaundice; early cirrhosis.
111	47	M	2950	12.7	11.2	265	494	1.97	10.2	Fatty liver; early cirrhosis; jaundice; ascites.
113	39	M	3500	4.97	3.40		334	2.08	2.1	Hemochromatosis; portal cirrhosis; diabetes; ascites.
112	65	M	1450	2.00	1.00		301	1.00		Portal cirrhosis; ascites.
39	62	M	3300	34.8	32.0		317	1.59	32.5	Fatty liver; periportal fibrosis and cirrhosis.
114	43	F	1600	26.4	24.4	172	246	1.59	24.5	Portal cirrhosis of the liver and ascites.
115	24	F	1700	3.98	2.97	208	263	2.00	1.68	Portal cirrhosis of the liver and ascites.
Average			2020	12.2	11.8	232	324	1.72	11.4	

TABLE IV  
*Cases of cirrhosis of the liver uncomplicated by alcoholism*

Case number	Age	Sex	Liver weight	Total lipids	Fatty acids	Cholesterol total	Phospho-lipids	Neutral fat	Pathology of liver and other disease processes
			grams	grams per cent	grams per cent	mgm. per cent	grams per cent	grams per cent	
31	38	M	1650	7.7	6.7	338	1.80	5.7	Lobar pneumonia; slight portal cirrhosis; central nervous system syphilis; calcified tuberculosis.
13	82	M	1300	4.63	2.84	284	1.39	2.00	Arteriosclerotic heart disease; lobular pneumonia; slight cirrhosis of the liver.
98	65	M	1600	3.27	2.05				Chronic passive congestion of liver and spleen; enlarged heart; cirrhosis of the liver.
38	55	F		5.30	4.20	227	1.33	3.4	Advanced nodular cirrhosis of the liver.
106	26	F	1880	3.82					Early cirrhosis of the liver; subacute pneumococcus aortic and mitral endocarditis; acute pneumococcus leptomenigitis.
Average			1557	4.94	3.95	283	1.51	3.7	

alcoholism. There was no elevation of the total lipid in this group, the highest total lipid being 7.68 grams per cent. The total cholesterol was done on 3 livers in this group and was within normal limits. If one assumes that cirrhosis of the liver is preceded by a period of fatty infiltration, these 5 cases should represent the end stage of the disease. Other factors, however, might account for the absence of fatty infiltration, *e.g.*, malnutrition or infection. Of these 5 cases, Case 31 had lobular pneumonia, central nervous system syphilis, cerebral cortical atrophy, and an old calcified tuberculosis. The state of nutrition in spite of these complications was fairly good. Case 13 had arteriosclerosis and lobular pneumonia; the state of nutrition in this patient was good. Case 98 had an enlarged heart with evidence of congestive heart failure; the state of nutrition was fair. Case 38 had no complications, but microscopic sections of the liver showed cords of dense fibrous tissue and a marked alteration in the lobular architecture of this organ; the diagnosis in this case was advanced nodular cirrhosis; the state of nutrition was good. Case 106 was in a poor state of nutrition and had subacute pneumococcus aortic and mitral endocarditis, and acute pneumococcus leptomeningitis; the liver showed early cirrhosis. In 3 of these 5 cases, the degree of cirrhosis of the liver was not advanced. Obviously, this is too small a group from which to draw any definite conclusions, but it seems as if cirrhosis of the liver may occur without previous fatty infiltration. In only one case was the nutritional state really poor.

#### DISCUSSION

The average total liver lipid in the group of 25 normal livers was 4.98 grams per cent. This agrees with other published observations on human livers and is about the same as the values reported for other mammalian livers. In tissue sampled as this was at varying periods after death, approximately 45 per cent of the total lipid was neutral fat. This is somewhat higher than is found in fresh specimens of dog liver where the neutral fat is approximately 15 to 20 per cent (1).

In the group of patients with chronic alcoholism, fatty infiltration occurred in 50 per cent of the livers. This observation has been made

before but chemical analyses give more definite evidence as to the varying degrees and the character of the fatty changes. Comparison of the individual and average figures in Tables II (normal) and III (alcoholic) shows changes in lipid distribution characteristic of fatty infiltration of the liver, *viz.*, increase in total lipid due to accumulation of neutral fat, increase in cholesterol esters and a decrease in the concentration of phospholipids. The last may be a dilution phenomenon due to the increase in the size of the liver. The total amount of phospholipid in the entire liver is actually greater in the alcoholic than in the normal group.

The phospholipid values in the normal group cover a wider but lower range than was previously found in normal dogs on a high calorie, high vitamin diet (1). Apart from differences in the nutritional history, this finding is probably due in part to the postmortem action in the human livers of esterases which split part of the phospholipids so that the fragment containing the lipid phosphorus is no longer soluble in petroleum ether, while the fatty acid moiety remains soluble (Table I). Hence, neutral fat values are apparently increased at the expense of phospholipids. Theis (6) also observed a higher ratio of neutral fat to phospholipids in normal human livers sampled at various intervals postmortem than is commonly found in normal animal livers that are analyzed shortly after excision.

Examination of Table I shows that there is approximately a 21 per cent decrease in the apparent phospholipid value within 24 hours after excision of the normal dog liver. If one assumed that an equal decrease occurred in these human livers and corrected for this decrease, the ratio would then approach that found in fresh tissue.

Observations such as these do not unfortunately shed any light on the mechanism of the production of this fatty infiltration of the liver in patients with histories of chronic alcoholism. Discussion of the etiology of this disorder must still rest on experimental data. As mentioned before, fatty infiltration of the liver can be produced experimentally in dogs by depancreatization, by ligation of the pancreatic ducts and by feeding an extreme high fat diet (14). *It can be prevented* in depancreatized or duct-ligated dogs by feeding raw

pancreas or choline or by feeding water-extracted meat powder in place of raw meat. In addition, Dragstedt (15) has reported a pancreatic hormone (lipocaic) capable of preventing fatty infiltration of the liver but the hormonal nature of the extract used has not as yet been confirmed by other investigators. Whether or not the pancreas is involved in the production of the fatty liver in the alcoholic patient is purely a matter of speculation. Connor (16) has advanced the hypothesis that fatty infiltration of the liver in chronic alcoholism is associated with a disturbance of carbohydrate metabolism. This disturbance, he thinks, may be due either to carbohydrate depletion of the liver or to an interference with the normal utilization of carbohydrate.

The fact that all patients with histories of chronic alcoholism do not develop fatty livers suggests that alcohol alone may not be the sole factor responsible, but the question of why some alcoholic subjects develop this condition while others do not remains a problem that requires further study.

#### SUMMARY

The livers of 25 normal subjects, 25 alcoholic patients and 5 patients with no history of alcoholism but with cirrhosis of the liver were analyzed for their lipid content. The total lipid content of the normal human livers varied from 2.42 to 8.41 grams per cent. The average total cholesterol was 283 mgm. per cent. Of the 25 alcoholic patients 13 had liver lipids above 9 grams per cent. The average total cholesterol was above 300 mgm. per cent. The total liver lipid was not elevated in the patients with cirrhosis of the liver who were not alcoholics.

We are indebted to Dr. Douglas Symmers, Director of the Department of Pathology of Bellevue Hospital, and to the Department of Hospitals and the Office of the Chief Medical Examiner for the samples of liver obtained for analysis.

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# THE RENAL BLOOD FLOW IN COARCTATION OF THE AORTA

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Arterial hypertension in the upper extremities has long been recognized as a frequent accompaniment of coarctation of the aorta (1), and has been produced experimentally by constriction of the aorta above the level of the origin of the main renal arteries in rats (2, 3) and in dogs (4, 5, 6, 7).

Pickering (8), studying the peripheral resistance in hypertension, and using a method which measured blood flow through heat elimination from the hand, examined three cases of coarctation. In these, as in all other examples of persistent hypertension which he studied, an abnormally high resistance to the flow of blood continued after influences from the nervous system had been released. Thus, pressor effects mediated through the nerves were excluded as a major cause of the increased peripheral resistance. Pickering believed that "a chemical abnormality of the blood" could be excluded also, because of the differences in blood pressure readings between the upper and the lower extremities. He concluded, by exclusion of the factors mentioned, that the vascular narrowing in the upper limb was accounted for best by a local change in its vessels.

The findings of other investigators are significant in relation to these conclusions. Lewis (9), and Blumgart, Lawrence and Ernstene (10) have shown that the blood supply is normal to both the upper and the lower extremities in patients with coarctation. Graybiel, Allen and White (11) found no evidence of muscular hypertrophy in the small arteries of the upper limb in coarctation.

Prinzmetal and Wilson (12) also studied the effect of the release of vasoconstrictor nervous influences in hypertension by means of the plethysmograph. They measured blood flow through the forearm under conditions of increased temperature, and in some cases following the injection of novocain into the upper dorsal sympathetic ganglia. They concluded that the hypertension

found in coarctation of the aorta was caused by a neurogenic increase in the peripheral vascular resistance. They were not in agreement with Pickering in this respect. For technical reasons, however, they did not attempt novocain injection into the dorsal sympathetic ganglia of patients with coarctation of the aorta.

In the experimental animal, Goldblatt and his associates (4, 5) demonstrated that constriction of the abdominal aorta just above the origin of the main renal arteries had little or no immediate effect on the blood pressure above the site of the clamp; the immediate effect below the clamp was a lowering of blood pressure. In about 24 hours, however, it was observed that a hypertension developed above the site of the clamp. Further, it was found that with elevation of the carotid blood pressure there was a concomitant rise above normal in the femoral artery pressure, despite a substantial constriction of the aorta.

Likewise, Steele (6) found that constriction of the aorta above the renal arteries in dogs resulted in hypertension both in the femoral and in the carotid arteries. He also found, by direct measurement, a definite diastolic hypertension in the femoral artery of a patient with coarctation (13). Page (7), studying the blood pressure in dogs in which the aorta was constricted above the diaphragm, noted little or no hypertension developing proximal to the occlusion but he found a hypotension distal to the occlusion which tended to return toward normal levels. However, if the aorta also was constricted below the origin of the renal arteries, "hypertension of renal origin" developed. Page believed that the inability to produce a sustained hypertension by constriction of the thoracic aorta was due to the development of sufficient collateral circulation to insure the prevention of substantial renal ischemia.

The reports quoted above make it clear that, in the experimental animal, constriction of the

abdominal aorta above the renal arteries is capable of producing systemic hypertension of renal origin. Constriction of the thoracic aorta in dogs, however, was followed by equivocal effects on the arterial pressure.

In humans, moreover, there has not been agreement as to the mechanism of the hypertension associated with coarctation of the aorta, nor has the important factor of renal ischemia been determined in these patients. In this communication, measurements of the effective renal blood flow in six cases of congenital coarctation of the aorta are reported.

#### METHODS

The work of Smith and his associates (14) has made available the technique of measuring the "effective renal blood flow" by means of the clearance of diodrast by the kidneys from the circulating plasma. The determination of both the effective renal blood flow and the rate of glomerular filtration in the six cases of coarctation studied was accomplished by the employment of both diodrast and inulin in the same general manner as described by these authors.<sup>1</sup>

Briefly, a preliminary priming infusion of diodrast and inulin in normal saline solution was given for 10 minutes and was followed by a sustaining solution of the same chemical in normal saline solution in lesser concentration. The iodine level of the blood was maintained at 0.5 to 2 mgm. per cent, and the inulin concentration varied from 40 to 60 mgm. per cent. Thirty minutes were allowed to elapse after the initiation of the sustaining infusion before urine collections were made. There were four urine collections, each of 20 minutes' duration. A blood sample was taken at the beginning, middle and end of each one. All tests were made on fasting patients in the recumbent position. Urine was obtained by catheter.

The urine and blood samples were analyzed for iodine according to the technique reported by White and Rolf (15). The inulin content of the urine and blood samples was determined by the colorimetric method described by Alving and his associates (16).

The renal plasma flow and the glomerular filtration were also determined from these specimens. The total renal blood flow was calculated by the addition of the volume of red cells, as obtained by hematocrit readings. All reported quantities are corrected to 1.73 square meters.

#### CASES

Six patients with definite coarctation of the aorta were studied. Brief abstracts are given below which present only the positive data establishing the diagnosis of coarctation.

<sup>1</sup> In addition, similar measurements were made on eleven individuals without evidence of any renal or circulatory abnormality.

#### *Case 1, W. D., male, age 52 years*

Blood pressure: brachial (right) 230/85; popliteal (right) 135/110. Left ventricular enlargement. Pre-cordial and interscapular systolic murmur. Normal retinal arteries. Palpable intercostal artery pulsations. Femoral artery pulsations weak and retarded. Scalloping of lower rib margins by x-ray. Urinary findings normal except for 1 to 2 erythrocytes per high dry field.

#### *Case 2, J. G., male, age 7 years*

Blood pressure: brachial (right) 140/90; brachial (left) 140/75; popliteal, not obtainable. Left ventricular enlargement. Systolic apical and interscapular murmur. Retinal arteries normal. Intercostal artery pulsations palpable. Femoral artery pulsations not palpable. Roentgenologic evidence of rib margin erosions and narrowing of the aortic arch. Urinary findings negative.

#### *Case 3, F. P., male, age 9 years*

Blood pressure: brachial (right) 305/110; (left) 172/105; popliteal (right) 134/?, (left) 106/?. Left-sided cardiac enlargement. Retinal arteries spastic and sclerotic. X-ray evidence of scalloped rib edges. Aortic arch not visualized by x-ray. Urinary findings negative.

#### *Case 4, M. J., female, age 33 years*

Blood pressure: brachial (right) 240/100; (left) 225/100; popliteal (left) 140/90. Spastic retinal arteries. Left ventricular enlargement. Soft interscapular systolic murmur. Palpable intercostal artery pulsations. Femoral artery pulsations retarded and barely palpable. Roentgenologic evidence of rib margin erosion and narrowing of the aortic arch. Urinary findings negative.

#### *Case 5, F. H., male, age 37*

Blood pressure: brachial (right) 210/105; (left) 205/100; popliteal not obtainable. Left ventricular enlargement. Interscapular systolic murmur. Retinal arteries slightly spastic. Femoral artery pulsations not palpable. Roentgenologic evidence of aortic arch narrowing and erosions of the inferior margins of the ribs. Urinary findings negative.

#### *Case 6, A. T., female, age 41 years*

Blood pressure: brachial (right) 220/110; (left) 210/110; popliteal (right) 130/?. Left ventricular enlargement. Palpable intercostal artery pulsations. Femoral artery pulsations not palpable. Urinary findings negative.

Thus it will be seen that these patients, of whom four were males and two females, and whose ages ranged from 7 to 52 years, in each case presented definite clinical and x-ray evidences of aortic coarctation.

#### RESULTS

Determinations of effective renal blood flow and inulin clearance were performed in eleven sub-

jects without circulatory or renal disturbances. This group was composed of six men and five women. The ages of the men ranged from 22 to 50 years, and the women from 25 to 40. The renal blood flows, inulin clearances, and filtration fractions in the six male subjects are tabulated in Table I-A. The renal blood flow averaged 1288

TABLE II

*Renal clearance determinations in coarctation*

## A. MALE

Case number	Age	Blood pressure (brachial)	Renal plasma flow	Hematocrit	Effective renal blood flow	Renal inulin clearance	Filtration fraction
		mm. Hg	cc. per minute	per cent serum	cc. per minute	cc. per minute	per cent
1. W. D.	52	230/85	412	53	775	118.5	28.8
2. J. G.	7	140/95	531	63	840	121.0	22.8
3. F. P.	9	172/105	445	60	742	129.5	29.0
5. F. H.	37	210/95	470	57	825	119.0	25.3
Average			464.5	58	795	122	26.5

## B. FEMALE

4. M. J.	33	240/100	370	62	595	121.0	32.7
6. A. T.	41	220/110	488	67	727	110.0	22.5
Average			429	64	661	115.5	27.6

TABLE I

*Clearance determinations in normal individuals*

## A. MALE

Case number	Age	Blood pressure (brachial)	Renal plasma flow	Hematocrit	Effective renal blood flow	Renal inulin clearance	Filtration fraction
		mm. Hg	cc. per minute	per cent serum	cc. per minute	cc. per minute	per cent
1. P. L.	22	130/80	815	57	1285	110	15
2. G. S.	25	110/75	573	63	910	122.5	22.7
3. S. B.	25	120/75	987	60	1640	128	12.9
4. A. E.	47	120/68	667	59.5	1125	137	20.5
5. S. L.	50	120/70	764	55	1390		
6. J. N.	50	110/70	760	55	1380		
Average			761	58	1288	124.4	17.8

## B. FEMALE

7. E. H.	25	95/75	695	70	990	153	22
8. L. D.	28	105/70	582	68	855	117	20.1
9. M. C.	30	118/70	575	71.5	805	124.5	21.6
10. N. C.	40	125/78	710	68	1045	116.5	16.4
11. E. M.	30	95/70	787	64	1230	118	15
Average			669	68.3	986	125.8	19.0

cc. per minute (range 910 to 1640). The average inulin clearance was 124.4 cc. per minute (range 110 to 137), and the average filtration fraction was 17.8 per cent (range 15.0 to 22.7). The clearance values for the five women studied are given in Table I-B. It was found that the renal blood flow averaged 986 cc. per minute (range 805 to 1230). The average inulin clearance was 125.8 cc. per minute (range 116.5 to 153) and the average filtration fraction was 19.0 (range 15 to 22.0). These values are similar to those obtained by Smith and his associates (17) in normal men and women.

The clearance measurements obtained in the six cases of coarctation are shown in Table II. The average effective renal blood flow in the four male cases was 795 cc. per minute (range 742 to 840), and 661 cc. per minute (range 595 to 727) in the two females. Thus, in all six cases, the renal

blood flow was markedly less than the average normal flow and in each one was lower than the smallest flow recorded in any normal individual studied. In these six cases, then, substantially reduced renal blood flow was demonstrated. However, the inulin clearances of these six cases were within the normal limits. As a result of the maintenance of a normal inulin clearance despite the reduction in renal blood flow, the filtration fraction was high, averaging 26.5 per cent (range 22.8 to 29.0) in the males, and 27.6 per cent (range 22.5 to 32.7) in the females.

## DISCUSSION

It is of prime interest that each of the six cases of coarctation showed a partial renal ischemia, as demonstrated by definite reduction in diodrast clearance. The demonstration of this ischemia does not necessarily prove the renal etiology of the hypertension present in these cases. In view of the fact that Lewis (9) found that the hypertension in aortic coarctation could not be explained on the basis of anatomical obstruction to aortic blood flow, and in view of the fact that hypertension occurs in dogs and in rats when the abdominal aorta is constricted above the renal arteries, it appears highly possible that the renal ischemia found in these six cases was the initiating factor in the production of the hypertension present.

It seemed quite important to us that, although these six patients showed a significant reduction in the blood flowing through the kidneys, the rate of glomerular filtration, as measured by inulin clearance, remained essentially normal. This maintenance of a normal inulin clearance, despite the reduction in the diodrast clearance, can be explained adequately only by the assumption that there is an increase in the intraglomerular pressure which, in turn, must be caused by an increase in the resistance provided by the glomerular efferent arteriole (18).

In cases of essential hypertension, also, it has been reported (18, 19) that there is a reduction in renal blood flow, but the glomerular filtration rate may be normal. These findings have led investigators to stress the important rôle played by spasm of the glomerular efferent arteriole in this disease. Various pathological studies (20, 21, 22) have demonstrated, however, that the organic obstruction to blood flow present in the kidneys of hypertensive patients is typically proximal to the glomerular tuft. Because it is impossible at the present time to determine in cases of essential hypertension the exact onset of the renal arteriosclerosis found at autopsy, it is difficult to ascertain whether the arteriosclerosis causes a reduced renal blood flow and a secondary compensatory spasm of the glomerular efferent arteriole, or whether the reduction in renal blood flow occurs because of a primary spasm of the efferent arteriole.

In the six cases herein reported, however, it is almost certain that the reduction in renal blood flow is due to the congenital aortic atresia and, although glomerular efferent arteriolar spasm is also present, it seems clear that the latter is distinctly secondary and compensatory to the afferent reduction in renal blood flow. Otherwise one must assume that these patients were born not only with aortic atresia but also with efferent arteriolar spasm, a most unlikely possibility. This secondary glomerular efferent arteriolar spasm present in these cases of coarctation cannot be considered as indubitable evidence that the efferent arteriolar spasm present in essential hypertension is likewise secondary in nature, but it does indicate that such spasm *can occur* following a reduction of the amount of blood flowing to the glomerulus.

## SUMMARY

1. The effective renal blood flow and the rate of glomerular filtration were measured by means of the diodrast and inulin clearances, respectively, in a group of eleven normal control subjects and in a group of six patients with coarctation of the aorta.

2. The findings in the cases of coarctation indicated an appreciable decrease in renal blood flow as compared to the normals. The glomerular filtration rate, however, was normal.

3. The arterial hypertension in coarctation is interpreted in the light of primary reduced renal blood flow associated with secondary glomerular efferent arteriolar spasm. A probable relationship of these factors to the pathogenesis of essential hypertension is pointed out.

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# MEASUREMENTS OF BLOOD FLOW AND BLOOD PRESSURE IN CLUBBED FINGERS

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Studies on the peripheral circulation in patients with clubbed fingers were reported in a previous communication (1). The maximum heat elimination from the finger tip was found to be increased and the digital arterial pressure elevated above normal in various pulmonary conditions and in one case of congenital heart disease with cyanosis. In several cases of unilateral clubbing the results varied, and in two cases of hereditary clubbing normal brachial-digital arterial pressure gradients were found.

It is the purpose of this communication to report changes in method which have made possible the measurement of finger tip blood flow in cc. per minute and to present further observations on a series of patients with normal and on a series with clubbed fingers. Twelve normal subjects, one patient with essential hypertension, one with aplastic anemia, one with hyperthyroidism, two with acromegaly, two with rheumatoid arthritis, two with chronic pyelonephritis, one with renal and genital tuberculosis and one with chronic salpingitis were studied in the first series. The second series included two cases of hereditary clubbing, seventeen cases of various pulmonary diseases, two of congenital heart disease with cyanosis, seven of subacute bacterial endocarditis, three of ulcerative colitis, one of bacillary dysentery, one of regional ileitis and two of sprue, all with clubbed fingers. In three of the pulmonary cases, there was hypertrophic osteoarthropathy. One case of lung abscess was studied both before and after operation.

## DISCUSSION OF METHOD

Stewart's method (2) for measuring hand blood flow was based on the principle that the amount of heat eliminated by the hand per minute in a water calorimeter is proportional to the volume flow of blood. Since the specific heat of the blood is approximately 1.0, he concluded that each cc. of blood flowing through the hand would re-

lease one calorie of heat to the calorimeter, provided that the temperature of the venous blood were  $1^{\circ}$  C. less than that of the arterial blood. If, therefore, the temperature of the venous blood were  $n^{\circ}$  C. lower than that of the arterial, each cc. of blood would release  $n$  calories to the calorimeter. The number of cc. of blood flowing through the hand per minute could therefore be determined from the number of calories eliminated per minute, divided by the difference in temperature between arterial and venous blood. Since the hand, in a water calorimeter set several degrees below mouth temperature, was cooled approximately to the temperature of the calorimeter, Stewart assumed the temperature of the venous blood to be equivalent to the average calorimeter temperature. He also found mouth temperature to be equivalent to the temperature of the arterial blood and thus presented a formula whereby the blood flow through the hand could be determined.

It was found, however, by Harris and Marvin (3) that the actual temperature of the venous blood from the wrist, with the hand in the calorimeter, was from  $1.4^{\circ}$  to  $4.0^{\circ}$  C. higher than that of the calorimeter after five minutes' immersion and from  $1.1^{\circ}$  to  $2.7^{\circ}$  C. higher after fifteen minutes. This invalidated the formula.

Pickering (4) nevertheless considered the correspondence between heat elimination in calories per minute and blood flow sufficiently close to attempt to establish normal values with a standardized procedure consisting of warming the body before each observation. This was known to release sympathetic tone almost completely and thus eliminated fluctuations in blood flow caused by variations in blood vessel caliber.

In order to study clubbed fingers, the Stewart method as modified by Pickering was adapted to the finger tip (1). A very much narrower normal range of variation in heat elimination per unit of tissue, after release of sympathetic tone, was found for the finger tip than had been noted by Pickering for the hand.



It was therefore thought that Stewart's formula, as presented below in METHOD, might have been found invalid for the hand because of a considerable indeterminate proportion of blood flow from the deep tissues. It might, however, be applicable to the finger tip where nearly all the blood flows through the surface capillaries and arteriovenous anastomoses. To test this, the temperature of the digital venous blood was measured in four subjects, as follows:

Sympathetic tone was released by immersing the left arm in water at a temperature of from 43° to 45° C. until sweating began. The entire fourth finger of the right hand was then immersed in an open calorimeter cup filled with water at an initial temperature of slightly less than 31° C. After two to five minutes of stirring, a hypodermic needle thermocouple was inserted into a vein on the dorsum of the base of the finger. The temperature of the venous blood was recorded at the same time as that of the calorimeter.

TABLE I

*Simultaneous temperatures of calorimeter and digital venous blood after release of sympathetic tone in four subjects*

	Temperature of venous blood	Temperature of calorimeter
	degrees centigrade	degrees centigrade
1	31.8	30.9
2	31.8	31.0
3	31.5	31.0
4	31.6	31.4

The results are presented in Table I. The temperature of the venous blood was from 0.2° to 0.9° C. (average 0.6°) higher than that of the calorimeter. The temperature of the arterial blood (mouth temperature<sup>1</sup>) was approximately 6.0° C. higher than that of the calorimeter. It is probable, therefore, that about 90 per cent of the blood flowing through the entire finger was cooled to the temperature of the calorimeter, the remaining 10 per cent coming from the deep tissues. It should be noted that this proportion applies to the entire finger. In the terminal phalanx, there is a greater proportion of surface to volume and a proportionately greater number of arteriovenous anastomoses than in the entire finger. If, therefore, it were possible to measure venous blood

temperature in the terminal phalanx, it would undoubtedly indicate that nearly 100 per cent of the blood flowing through the finger tip was cooled to the temperature of the calorimeter. These observations demonstrate the accuracy of Stewart's formula when used for the measurement of blood flow in the finger tip.

The normal range of variation in finger tip volume flow was between 0.21 and 0.29 cc. per sq. cm. of finger tip surface per minute and between 0.57 and 0.97 cc. per cc. of finger tip per minute. The latter is in accord with the range found by Wilkins, Doupe and Newman (5) (0.6 to 1.2 cc. per cc. per minute). Their measurements were made with the finger plethysmograph on the terminal two phalanges after release of sympathetic tone by warming the body. For the purposes of this investigation the calorimetric method was preferable to the plethysmographic for several reasons. The apparatus of the former is simpler and can be used at the bedside. The small degree of distensibility of the vascular bed of the finger tip, moreover, makes it difficult to measure blood flow by the plethysmographic method in the terminal two phalanges and almost impossible in the terminal phalanx. It should be noted also that the range of variation per unit surface was considerably narrower than per unit volume. This was due, in part at least, to inaccuracy in the measurement of finger tip volume by water displacement.

The measurement of pressure gradients presented comparatively fewer problems. The brachial arterial blood pressure was measured by the standard auscultatory method and the digital arterial blood pressure with the Gaertner capsule (6), as already described (1). When sympathetic tone had been released it was usually possible to check these pressures within a few mm. Hg. Variations in systolic and diastolic pressures, when both were measured, made it impossible to determine representative brachial-digital gradients. These gradients could be standardized physiologically only if it were clinically feasible to determine true mean pressures. An approximation, however, to the physiological mean pressure gradient was adduced from the arithmetic mean pressures. The normal range of variation in arithmetic mean pressure gradient after release of sympathetic tone was from 9 to 40 mm. Hg.

<sup>1</sup> The close correspondence found by Stewart (2) between mouth temperature and the temperature of the arterial blood measured indirectly was confirmed by direct thermocouple measurement of arterial blood temperatures.

## METHOD

Each observation was carried out as follows: With the subject sitting comfortably in bed in a room kept at a fairly constant temperature with minimal air currents, preliminary mouth temperature, pulse rate, and brachial and digital arterial blood pressures were recorded. The left arm was then immersed in a water bath which was continually stirred and the temperature of which was maintained between 43° and 45° C. After ten to twenty minutes, when release of sympathetic tone was almost complete, as indicated by the onset of generalized sweating, the fourth finger tip of the right hand was inserted into the water calorimeter set at approximately 6.5° C. below mouth temperature. The calorimeter was stirred and the rise in temperature per minute recorded for several minutes until the total rise was about 1° C. The finger tip was removed from the central opening of the calorimeter and a small cork inserted. The fall in calorimeter temperature was recorded for several minutes. Mouth temperature, pulse rate, and brachial and digital arterial blood pressures were again measured, following which the left arm was removed from the water bath. The surface area and the volume of the previously immersed finger tip were measured by strips of adhesive tape and water displacement, respectively.

Blood flow per unit surface of finger tip was calculated from the following formula:

$$F = \frac{(\Delta t_1 + \Delta t_2)(m + e)}{sa(t_2 - t_1)},$$

in which  $F$  is the blood flow in cc. per sq.cm. per minute;  $\Delta t_1$ , the rise in temperature of the calorimeter per minute;  $\Delta t_2$ , the fall in temperature of the calorimeter per minute after removal of the finger tip;  $m$ , the volume of water in the cup;  $e$ , the hydrothermic equivalent of the cup, stirring device and thermometer bulb;  $s$ , the specific heat of the blood (approximately 1.0);  $a$ , the area of the immersed finger tip in sq.cms.;  $t_2$ , the temperature of the arterial blood (mouth temperature); and  $t_1$ , the temperature of the venous blood (average calorimeter temperature). To calculate the blood flow per unit volume, the volume of the finger tip in cc.,  $v$ , is substituted for  $a$  in the formula.

## RESULTS

The results are presented in Tables IIA and IIB. In the cases of hyperthyroidism, aplastic anemia, essential hypertension and acromegaly, the blood flows and brachial-digital arterial pressure gradients were within normal limits. In the cases of chronic infection without clubbing, such as rheumatoid arthritis, pyelonephritis, renal tuberculosis, and salpingitis, the blood flows and brachial-digital arterial pressure gradients were also within the normal range.

TABLE II A  
Normal fingers

Diagnosis	Blood flow cc. per sq. cm. per minute	Blood flow cc. per cc. per minute	Brachial arterial pressure mm. Hg	Digital arterial pressure mm. Hg	Arith- metic mean gradient mm. Hg
Normal.....	0.25	0.69	128/80	120/70	9
Normal.....	0.24	0.59	102/74	88/40	24
Normal.....	0.22	0.62	125/84	96/52	30
Normal.....	0.28	0.93	135/94	110/76	22
Normal.....	0.23	0.62	114/82	106/52	19
Normal.....	0.28	0.76	132/86	116/60	21
Normal.....	0.27	0.72	112/82	84/70	20
Normal.....	0.28	0.97	128/80	102/60	23
Normal.....	0.23	0.57	118/85	102/64	18
Normal.....	0.29	0.80	118/90	96/60	26
Normal.....	0.26	0.74	116/86	98/54	25
Normal.....	0.21	0.65	124/90	100/35	40
Aplastic anemia....	0.20	0.60	112/70	96/64	11
Hyperthyroidism....	0.22	0.73	130/78	100/84	12
Hypertension.....	0.20	0.66	238/125	216/130	9
Acromegaly.....	0.22	0.54	108/86	104/?	
Acromegaly.....	0.21	0.48	120/78	108/64	13
Rheumatoid arthritis.	0.28	0.88	120/80	100/76	12
Rheumatoid arthritis.	0.26	0.87	106/74	90/74	8
Chronic pyelonephritis....	0.24	0.89	186/92	142/98	21
Chronic pyelonephritis....	0.29	1.00	140/82	128/64	15
Tuberculous nephritis	0.24	0.76	128/98	108/70	24
Chronic salpingitis...	0.23	0.81	104/50	66/32	28

In the cases of clubbed fingers secondary to various pulmonary diseases, the blood flows were usually above the upper normal limit, although in a few cases they fell within the normal range. The brachial-digital arterial pressure gradients in the cases of simple clubbing secondary to pulmonary disease were generally less than normal because of elevated digital arterial pressures. In the three cases of hypertrophic osteoarthropathy, the blood flows were within normal limits and the pressure gradients only slightly or moderately less than normal. In one of the cases of lung abscess, the blood flow before operation was above normal and the brachial-digital arterial pressure gradient less than normal because of elevated digital arterial pressure. After incision and drainage there was a partial recession of the clubbing. The blood flow decreased to within normal limits and the digital arterial pressure was lower.

In the two cases of congenital heart disease with cyanosis, the blood flows were high normal in one and above normal in the other. The pressure gradients were less than normal in both. In five of the cases of subacute bacterial endocarditis, the



# THE TOXICITY OF ORALLY ADMINISTERED POTASSIUM SALTS IN RENAL INSUFFICIENCY<sup>1</sup>

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(Received for publication August 2, 1940)

Smillie (1) in 1915 reported the sudden collapse of a patient with nephritis seven hours after the oral administration of 10 grams of potassium chloride. He attributed this collapse to an excessive accumulation of potassium in the body owing to impaired excretion. The patient ultimately recovered. Smillie also found that sudden death commonly followed the oral administration of potassium salts to rabbits with uranium nephritis. Similar amounts were without effect in control animals. He reported no chemical determinations of potassium in the serum or urine. Smillie's observations have frequently been cited as indications of the possibility of potassium poisoning in renal insufficiency. However, the complex extra-renal changes which occur in uranium nephritis and the peculiarly specialized character of the rabbit kidney make it improper to generalize from these experiments concerning oral potassium poisoning. There is, on the other hand, reason for minimizing the danger of toxicity from potassium in human nephritis. High concentrations of potassium in the serum are rare even in the most advanced nephritis (2), although these patients may ingest considerable amounts of meat and other foods containing potassium, and although they must continually be liberating potassium from the breakdown of tissue.

In the present study an attempt is made to ascertain whether humans or animals can be poisoned by oral administration of potassium. Potassium salts were administered to human subjects with and without renal impairment, and the concentrations of potassium in serum and urine followed at intervals thereafter. In another type of experiment, dogs whose ureters had been tied received potassium salts by stomach tube. Frequent electrocardiographic observations and chem-

ical determinations of potassium in serum were used for the detection and estimation of toxic effects of potassium in these animals (10).

## METHODS

### (A) General

The method of conducting the observations in fasting human subjects has been described elsewhere (3). Samples of urine and of serum were obtained at intervals before and after the ingestion of potassium salts. Urine specimens were collected without catheterization. Usually considerable water was given during the course of the experiment. Potassium salts were administered either in capsules, followed by water, or dissolved in tomato juice. Urea clearances were determined in all experiments, and in some, sucrose clearances were measured after preliminary intravenous injections of sucrose. Both sucrose and urea clearances vary with the glomerular filtration rate and in renal disease are reduced in about the same proportion as the filtration rate (3, 4). Accordingly, they may both be used as relative measures of glomerular filtration. Potassium was determined as the chloroplatinate by the method of Hald (5). Clearances were calculated by the general method previously described (3).

For the animal experiments nine dogs were employed. All experiments were carried out under nembutal anesthesia (40 mgm. per kgm.). A tracheal cannula was inserted. Urinary excretion was suppressed by ligation of the ureters. Potassium chloride in isotonic or hypertonic solution was introduced by tube into the stomach or duodenum. Serial electrocardiograms from lead II were taken and samples of blood for potassium analysis were obtained by direct puncture of the heart at the termination of the experiment.

### (B) Electrocardiographic changes as criteria of potassium poisoning

As the concentration of potassium in the serum rises, the following sequence of electrocardiographic changes is observed (6): (1) increased amplitude of the T wave, (2) depression of the ST segment with development of a diphasic T wave, (3) disappearance of the P wave, (4) intraventricular block, and (5) cardiac arrest at concentrations of serum potassium of 12 to 20 m.eq. per liter. These changes, save for the last, are reversible. No one of them is, of course, characteristic; but when

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TABLE I

*Fasting excretion and clearance of potassium; no salts of potassium ingested*

Number	Diagnosis	Serum potassium		Urine potassium		Clearances		
		Time †	Concentration	Time	Excretion rate	Potassium	Sucrose	Urea
		min-utes	m. eq. per liter	min-utes	m. eq. per minute $\times 10^3$	cc. per minute	cc. per minute	cc. per minute
1	Arthritis			0-33 33-65 65-93	50.2 53.2 35.5	10.0* 10.6* 7.1*	143 122 122	40 76 60
2	Diabetes	45 128	5.00 4.60	0-42 42-95 95-128	53.9 70.9 43.4	10.8 14.2 9.0	111 110 108	42 40 41
3	Hyperthyroidism	112	5.15	6-71 71-115	47.3 36.9	9.2 7.4	170 126	67 55
4	Nephrosclerosis	8 133	5.00 5.00	0-37 37-81 81-129	42.8 46.9 32.8	8.6 9.4 6.6	92 82 68	47 36 30
5	Pyelonephritis	5 144	5.40 5.65	0-36 36-92 92-135	50.3 46.0 40.1	9.7 8.9 7.1	16 18 15	9 9 8

\* Assuming serum potassium of 5.0 m.eq. per liter.

† Time from beginning of experiment.

found in sequence they are sufficiently specific to serve as criteria by which the presence and degree of cardiac potassium poisoning may be detected.

## RESULTS

### (A) Observations on human subjects

Five experiments, with fasting human subjects, are summarized in Table I. In Subjects 1, 2 and 3 glomerular filtration, as measured by the sucrose and urea clearances (3, 4), was normal; in Subject 4 somewhat reduced, and in Subject 5 severely impaired. In all five, however, the fasting rate of excretion of potassium was approximately the same, averaging about 0.05 m. eq. per minute,<sup>2</sup> and varying over the extreme range of 0.03 to 0.07 m. eq. per minute. The excretion rate was no lower in Subject 5 than in the others. Since the serum concentration was normal in all experiments, this means that fasting potassium clearance was usually about the same in all subjects, whether

<sup>2</sup> The average fasting excretion rate of 0.05 m.eq. per minute corresponds to 2.9 grams of potassium daily. On the assumption that in the breakdown of tissue 1 gram of potassium is released for every 2.5 grams of nitrogen (2), this corresponds to  $2.9 \times 2.5 \times 6.25 = 45$  grams of protein broken down daily. This is a reasonable figure for tissue catabolism.

or not glomerular filtration was reduced. In magnitude it was about 10 cc. per minute; this was about one-twelfth or one-fifteenth of the average glomerular filtration rate in normal subjects (7), but amounted to nearly half the glomerular filtration in Subject 5. This means that in the subjects with impaired function a normal fasting excretion rate of potassium was maintained by a great decrease in the usual proportion of potassium per unit of filtrate reabsorbed by the tubules.

The response of two subjects with normal renal function to the ingestion of potassium salts is recorded in Table II A. In the first subject (1a) the rate of excretion of potassium increased over fivefold. The serum concentration rose but slightly, so that the clearance of potassium rose to an almost equal extent. In the second subject (2a) the increase is partially masked by an abnormally high fasting excretion rate of potassium, due to some unknown cause. Here too, however, an excretion rate many times the normal fasting value continued for some hours after ingestion and (since the serum concentration rose but little) was associated with a marked elevation of the potassium clearance. In spite of this increased excretion rate, elimination of the ingested potassium was slow. In Experiment 1a only about one-third was eliminated in two hours, while in Experiment 2b only about three-fourths had been recovered from the urine in five hours.

The course following the administration of potassium salts in five subjects (1b, 2b, 3b, 4b, 5b) exhibiting varying degrees of renal impairment is outlined in Table II B. In these subjects, too, the rate of excretion increased definitely following the ingestion of potassium, but not as much as it did in the normal subjects of Table II A. The maximal increase in potassium concentration in the serum was about the same in nephritic as in normal subjects but the elevated concentration persisted for a longer period in the more severe nephritics. Since the initial level was a little higher in some of the nephritic subjects, the final total concentration attained was sometimes a trifle higher. However, the concentration never exceeded 8 m. eq. per liter with doses of the size here given. Attempts to administer potassium salts in amounts greater than these were unsuccessful, since cramp-like epigastric pains followed by nausea and vomiting regularly intervened. In

TABLE II

(A) Subjects without renal impairment; response to ingestion of potassium salts

Number	Diagnosis	Potassium ingested		Serum potassium		Urine potassium			Total amount excreted	Clearances		
		Time	Amount	Time	Concentration	Time	Excretion rate	Amount excreted		Potassium	Sucrose	Urea
		minutes*	m. eq.	minutes*	m. eq. per liter	minutes*	m. eq. per minute $\times 10^3$	m. eq.†	m. eq.	cc. per minute	cc. per minute	cc. per minute
1A	Hypertension	0	67	3 68 127	4.75 5.75 5.50	0-42 42-80 80-124	45.6 244.0 238.0	1.9 9.3 10.5	21.7	8.7 42.3 42.8	139 208 207	65 94 103
2A	Hodgkin's disease	83-143	100	0 150 248 376	4.80 7.65 6.25 5.00	0-53 53-80 80-125 125-240 240-375	280.0 143.8 224.0 345.0 238.2	(14.8) (3.9) 10.1 39.7 32.1	81.9	58.0 30.1 29.3 50.5 42.2		157 91 95 61 56

(B) Subjects with renal impairment; response to ingestion of potassium chloride

1B	Nephrosclerosis	134	67	0 67 162 211	4.90 5.00 5.45 6.25	0-58 58-140 140-208 208-270	22.4 63.5 64.0 61.5	(1.3) 5.2 4.4 3.8	13.4	4.6 12.1 10.2 9.8	71 49 71	56 32 29
2B	Acute nephritis	105-115	67‡	0 207 246 296	6.25 6.50 5.90	0-95 95-202 202-240 240-270 270-292	45.2 79.3 114.3 74.1 68.9	(4.2) 8.5 4.4 2.2 1.5	16.6	9.0 14.1 18.0 11.4 11.3	36 25 23	20 22 26 16 15
3B	Acute nephritis	79-146	107	0 79 196 232 300 409	6.35 6.95 7.45 7.65 7.70 7.95	0-42 42-79 79-122 122-189 189-227 227-297 297-350 350-406	54.7 48.9 66.2 83.7 104.5 87.0 114.0 89.9	(2.3) (1.8) 2.9 5.6 4.0 6.1 6.0 5.0	29.6	8.6 7.9 10.0 11.6 13.9 11.3 14.9 11.5		9 10 13 9 8 6 12 7
4B	Chronic nephritis	106-146	134	0 180 357	4.90 6.75 7.35	0-48 48-104 104-135 135-174 174-251 251-301 301-357	32.5 — § 49.0 73.7 — § 84.0 93.3	(1.6) 2.2 estimated 1.5 2.9 6.2 estimated 4.3 5.2	22.3	8.6 9.5 12.5 12.2 12.9		9 8 9 9 11
5B	Chronic nephritis	156-241	94	84 150 238 326 404	5.00 6.05 6.70 6.95 7.50	0-90 90-155 155-234 234-322 322-401	41.3 75.8 99.2 110.3 112.0	(3.7) (4.9) 7.8 9.8 8.8	26.4	8.3 13.8 15.6 16.2 15.5		17 23 24 19 18

\* Time from beginning of experiment.

† Figures in parentheses are not included in the summation of the amount excreted.

‡ Vomited.

§ Collection incomplete.



Experiment 3b "collapse," similar to that described by Smillie, occurred. The patient became very pale, sweated profusely, the pulse became rapid, the blood pressure fell, and he lost consciousness for an instant. He then vomited a small amount of fluid after which the symptoms disappeared completely and he felt entirely normal. The serum potassium was 7.70 m. eq. per liter at the time of the attack, and continued to rise to 7.95 later, although the patient had recovered completely. This makes it unlikely that the episode was due to cardiac potassium poisoning. It appeared rather to have been a transient reflex vasodilatation in a subject with acute arterial disease, perhaps induced by gastro-intestinal irritation, *i.e.*, a vagotonic associate of nausea and vomiting.

Since the increase in the rate of excretion of potassium is less than that in normal subjects, elimination is correspondingly delayed, so that even after five and six hours only one-quarter or less of the ingested potassium had been eliminated. Nevertheless, as has been observed, the serum concentration is only moderately elevated. The reason for this presumably lies in the mode of distribution of potassium in the body. Also, in these cases its absorption may have been greatly delayed.

The increase in concentration of potassium in the serum following the ingestion (8) or injection (9, 10) of potassium salts is only about one-third as great as that which would be expected were its distribution purely extracellular. In other words, about two-thirds of the absorbed potassium leaves the extracellular fluid. This amount can be accounted for on the assumption that a uniform rise of potassium concentration of the same magnitude as that in serum water occurs throughout the cellular water. However, segregation of some or all of this potassium in some particular tissue is equally consistent with the facts. Hahn, Hevesy and Rebbe (11), using a radioactive isotope of potassium, have demonstrated an absence of diffusion equilibrium between cellular and extracellular potassium. This indicates that potassium ions exchange relatively slowly across the cell membranes of the body, and so perhaps makes the hypothesis of uniform distribution in all body water somewhat less probable. The interpretation of their experiments, in which the concentration

of radioactive potassium in various tissues was determined, is not entirely clear; possibly some special accumulation in bones took place. Whatever the mechanism, it is certain that the body does have the capacity to store at least two parts of potassium elsewhere for each part that accumulates in the extracellular fluid. This means that, with respect to potassium, the body has a buffer capacity by which increases in the concentration of this element in the serum, after ingestion of its salts, are minimized. The quantitative importance of this buffer mechanism may best be seen by considering a typical example. This has been done graphically in Figure 1, using data of Experiment

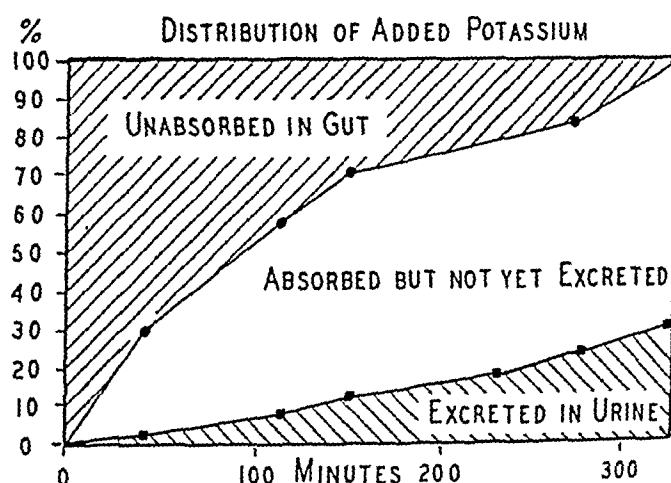


FIG. 1. PROPORTIONS OF THE INGESTED POTASSIUM (A) STILL UNABSORBED FROM GUT, (B) ABSORBED AND PRESENT IN THE BODY AND (C) EXCRETED IN THE URINE

The amount present in the urine was determined by chemical analysis. That present in the body was calculated on the assumption that one-third of the ingested potassium was present in the extracellular fluid. The increase in concentration of potassium in the serum water was multiplied by the volume of the extracellular fluid (assumed to be 15 liters), and this figure in turn multiplied by three to obtain the total amount distributed in the body. The amount still present in the gut was calculated by difference. Since the factor three is based largely on experiments with intravenous injections in dogs and in a very few human subjects, it is quite an approximate one. Therefore, these calculated values are only approximate, but serve to illustrate the several components in the system. From the data of Experiment 3b, Table II B.

3b of Table II B. Absorption from the gut is slow, being complete only after some five hours. Excretion in the urine is also very gradual. At the end of five hours the greater part of the potassium can be accounted for within the body. Be-

cause a large proportion finds a temporary repository outside the extracellular fluid, the increase in the concentration of potassium in serum water is only about one-third as great as that of a substance limited to an extracellular distribution.

Although excretion rate is not as greatly elevated in patients with renal insufficiency as in normal subjects, it increases sufficiently to cause the clearance of potassium to rise. This enables the excess potassium to be eliminated without a great change in the potassium of serum and, although elimination is slow, it is sufficiently increased above normal so that the ingested potassium could be eliminated within twenty-four hours.

#### *Discussion of the observations on human subjects*

The experiments with human subjects which have just been described indicate the reasons for the rarity of potassium poisoning in human nephritis. In summary they are as follows:

(1) The fasting clearance of potassium is not reduced in renal disease, so that the basal excretion of potassium continues without increase in potassium in the serum.

(2) The distribution of the potassium ion beyond the confines of the extracellular fluid provides a temporary storage site for large amounts of potassium without much increase in serum concentration.

(3) The relatively slow gastro-intestinal absorption of potassium prevents transient high concentrations of potassium in the serum.

(4) Even with the most severe renal disease the clearance of potassium rises significantly above the fasting level following the ingestion of potassium salts. The associated increase in excretion rate permits the extra ingested potassium to be eliminated after some hours.

(5) It is difficult to administer amounts of potassium too great to be cared for by the storage and excretory capacities without inducing nausea and vomiting.

However, the possibility that potassium poisoning may under some circumstances occur in renal insufficiency has not been excluded. Since experiments designed to test this possibility are obviously too dangerous to attempt with human subjects, resort was had to animal experimentation.

#### *(B) Experiments with dogs*

##### *(1) Control experiments.*

Potassium salts were introduced directly into the duodenum of two normal anesthetized dogs in order to insure the most rapid possible absorption. In spite of this, the potassium of serum rose but slightly after four to ten hours, and only the earlier electrocardiographic changes appeared. Rapid absorption from the gastro-intestinal tract certainly took place under these conditions, as was apparent in experiments similar in every respect save that the ureters were obstructed. Failure to produce a notable accumulation of potassium in the body fluids appears, therefore, to have been due to rapid excretion rather than faulty absorption. Evidently, the rate at which potassium may normally be excreted by the kidneys easily equals the maximal rate of absorption from the gastro-intestinal tract. It is therefore reasonable to conclude that fatal poisoning from oral ingestion of potassium cannot occur in the presence of normal renal function.

(2) Experiments with dogs with ligated ureters.

##### *(a) Delay in absorption of potassium salts introduced into the stomach.*

When solutions of potassium chloride were introduced into the stomach by tube, electrocardiographic or chemical evidences of potassium poisoning usually failed to develop for an hour or more. Pressure on the abdomen forced back through the stomach tube as much fluid as had been administered, indicating that the absence of potassium effects was due to failure of absorption. The cause for this was at once apparent on opening the abdomen. The pylorus was so tightly contracted that even with vigorous manual manipulation it was impossible to force the stomach contents past it into the duodenum.

The fluid was permitted to remain in the stomach of three animals. One animal showed the first electrocardiographic signs of potassium poisoning after thirty-five minutes, and died after an hour. The other two still survived at the end of ten hours, but with unmistakable electrocardiographic changes characteristic of pre-terminal potassium poisoning and with concentrations of potassium in the serum of 10.1 and 10.9 m. eq. per liter, respectively. It seems probable that the

long survival was due to cessation of absorption of potassium due to circulatory failure after the potassium concentration had risen to a level nearly, but not quite, sufficient to produce death. There is other evidence that such circulatory failure does occur (12).

(b) Duodenal administration of potassium.

Potassium chloride was administered by means of a tube passed through the pylorus via a gastrostomy in order to avoid delay in absorption due to pylorospasm. In three experiments this was done after a previous unsuccessful attempt

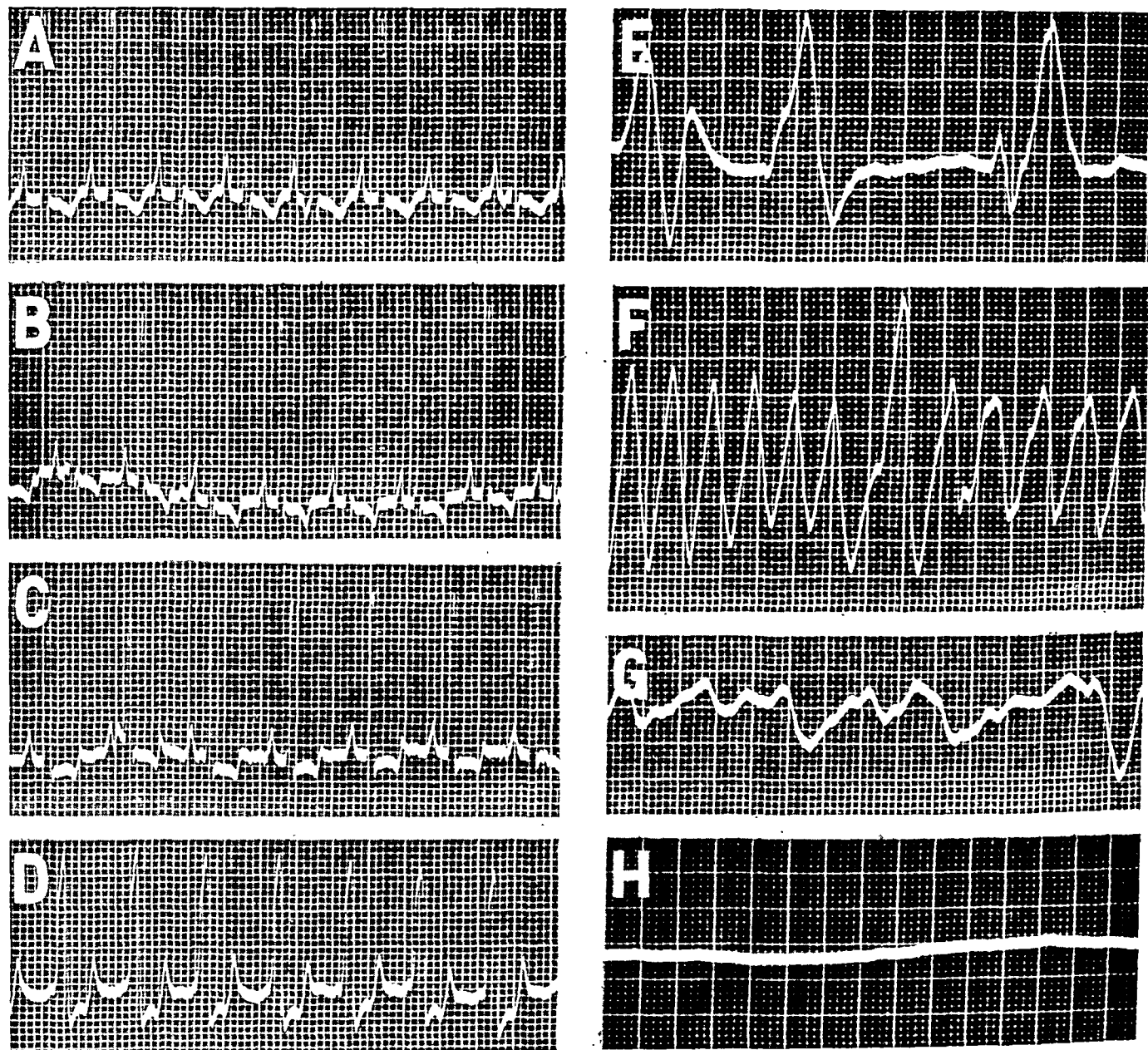


FIG. 2. SERIAL ELECTROCARDIOGRAMS OBTAINED IN A DOG WITH LIGATED URETERS FOLLOWING THE ADMINISTRATION OF TWICE ISOTONIC KCl SOLUTION BY TUBE INTO THE DUODENUM

All records from lead II. Dog, 7.5 kgm.

(A) Control.

(B) No change seventeen minutes after 1 liter of solution before ureters were tied.

(C) Depression of ST segment and beginning diphasic T wave fifteen minutes after ligation of ureters and administration of another liter of KCl.

(D) Disappearance of P waves, slight intraventricular block, marked ST segment depression, diphasic T wave thirty-five minutes after ureteral ligation.

(E) Marked intraventricular block and some slowing fifty minutes after ligation.

(F) and (G) Sudden ventricular flutter, followed within two minutes by coarse ventricular fibrillation.

(H) Arrest two minutes later. Concentration of potassium in serum 13.0.

to bring about absorption by introduction into the stomach, while in three others no such prior attempt had been made. Prompt and vigorous purgation occurred in all three experiments, and much of the solution administered was quickly lost in this way. All six animals died within a few minutes to an hour, depending on the rate at which the potassium chloride was administered. That death was due to cardiac potassium poisoning appears certain from the characteristic sequence of electrocardiographic changes in each instance (Figure 2, A to E), and from the high concentrations of potassium in the blood just before death. These were 9.8, 10.8, 12.4, 13.0, 13.0, 14.4 m. eq. per liter, respectively, and fall in or just below the range associated with cardiac arrest from intravenous potassium (6).

In a few of these experiments exitus did not occur following cardiac slowing and arrest. Instead, the rate was maintained for some time in the face of increasing intraventricular block until suddenly ventricular flutter and coarse fibrillation appeared (Figure 2, F, G). It seems reasonable to suppose that in these experiments circulatory failure occurred as the result of intraventricular block, delaying further absorption of potassium so that the final stages of slowing and arrest did not appear.

#### DISCUSSION

Evidently, it is possible for fatal poisoning from oral potassium to occur under certain experimental conditions. The essential conditions are suppression of urinary secretion and direct introduction of the potassium solution into the duodenum. In the dog, at least, this second condition is difficult of attainment after ordinary oral administration owing to pylorospasm. The relatively slow absorption of potassium and the severe epigastric pain, cramps and vomiting observed in human subjects after potassium salts are administered strongly suggest that in man, too, pylorospasm may occur. No pylorospasm occurred in control experiments in which water or normal saline was introduced into the stomach of a dog with ligated ureters. This spasm after potassium chloride is therefore not simply a response of the pylorus of such an animal to the ingestion of any fluid. However, there is no evidence that the

production of pylorospasm is a unique property of the potassium ion, as other irritant salts were not tested.

The animal experiments, therefore, indicate that, while oral potassium poisoning may occur, the conditions necessary for its production are not likely to occur in human subjects with nephritis. In a deeply anesthetized animal with complete anuria, enough potassium may eventually pass the pyloric sphincter to cause death. In man, comparable doses are, of course, not given; and even with smaller amounts vomiting acts to remove a great proportion of the material administered. Moreover, a distinction must be drawn between the situation in nephritis and that in complete anuria. In the nephritic patient renal function is rarely completely suppressed. The rate of excretion of potassium, though reduced, is sufficient to eliminate that coming from tissue breakdown, as well as a certain excess. Consequently, the small amounts passing the sphincter and absorbed from the duodenum can be gradually eliminated, while the distribution in the body minimizes the transient increase in concentration of circulating potassium. On the other hand, in complete anuria (such as that following mercury poisoning), there is obviously much more possibility of potassium poisoning, since the delays of absorption and the wide distribution are of temporary value only. Indeed, in animals with complete urinary obstruction, gradual elevation of serum potassium due to tissue breakdown is the rule (13). The danger of giving even small amounts of potassium by mouth under such circumstances is self-evident.

#### CONCLUSION

1. Cardiac death due to potassium absorbed from the gastro-intestinal tract can be produced experimentally in dogs whose ureters have been tied.
2. Direct introduction of potassium salts into the stomach induces spasm of the pylorus, preventing absorption and delaying death. Rapid death occurs when potassium salts are introduced directly into the duodenum of animals with ligated ureters.
3. It is unlikely that the conditions necessary for fatal poisoning by oral potassium administra-

tion can occur in patients with nephritis so long as urine is being excreted. The relatively slow absorption, the vomiting when large doses are given, the mode of distribution in the body and, most important of all, the continued ability of the most severely damaged nephritic kidney to excrete potassium all combine to make such poisoning very difficult to bring about.

4. Poisoning from oral administration of potassium is a more distinct possibility with complete anuria from any cause.

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# THE EFFECTS OF THE PATENT DUCTUS ARTERIOSUS ON THE CIRCULATION<sup>1</sup>

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The operation for ligation of the patent ductus arteriosus, as described by Gross and Hubbard (1), presented an opportunity to study the circulatory dynamics of this congenital anomaly. A patent ductus arteriosus is a vascular communication between the aorta and the pulmonary artery. Because the blood pressure is normally higher in the aorta than in the pulmonary artery, and because the arterial blood of patients with patent ductus arteriosus has been found to be saturated with oxygen to a normal degree, it has been assumed that in uncomplicated cases the flow of blood through the ductus is in the main from aorta to pulmonary artery. The amount of blood passing through the ductus per unit of time was calculated by Plesch in 1909 (2); however, he used a method which in the light of present knowledge is not valid.

The purpose of this paper is to report the results of quantitative studies of the circulation in patients with patent ductus arteriosus. The problem is further elucidated by measurements in animals in which a situation similar to a patent ductus arteriosus has been brought about by operation.

In normal individuals and in those with certain types of cardiac abnormalities the output of the heart may be determined by the foreign gas method of Marshall and Grollman (3), provided that samples of the foreign gas from the lung-bag mixture are obtained *after* equilibrium has been reached and *before* recirculation to the lungs has occurred. This method is not applicable to patients with patent ductus arteriosus because, when such patency is present, a portion of the foreign gas absorbed by the blood during its passage through the capillaries of the lung is almost immediately returned to the lungs through the ab-

normal vessel, making it practically impossible to obtain samples before recirculation occurs. This criticism appears to apply to other indirect methods also, such as the ethyl-iodide method of Starr and Gamble (4) or the oxygen-carbon dioxide method of Burwell and Robinson (5). The possibility of obtaining blood from aorta and pulmonary artery at the time of operation leads to a consideration of the applicability of the so-called "direct" method, depending upon the principle of Fick (6). This principle may be stated as follows: Given the amount of oxygen per 100 cc. of blood entering a given vascular area, the amount of oxygen added to or subtracted from the blood during its passage in a unit of time, and the amount of oxygen per 100 cc. of blood leaving the area, the volume of blood flow through it during that time may be calculated. The calculation is made according to the formula

$$C = \frac{O}{A - V}$$
in which  $C$  is the volume of flow (cardiac output) per minute,  $O$  is the oxygen absorbed per minute,  $A$  is the arterial oxygen content, and  $V$  is the venous oxygen content.

In people with no abnormal connection between peripheral and pulmonary circuits the volume of blood passing through the lungs is (essentially) the volume of blood put out by the right ventricle, which is necessarily the same as the volume put out by the left ventricle. Neither of these statements, however, need be true in a patient with a patent ductus arteriosus. As we shall show, the flow through an uncomplicated patent ductus arteriosus is from aorta to pulmonary artery and, therefore, blood expelled from the left ventricle may pass via the ductus and the pulmonary blood vessels back to the left ventricle without having passed through the right. Clearly, under these circumstances the output per minute of the left ventricle may be greater than that of the right (Figures 1 and 2).

<sup>1</sup> Read in part at the thirty-second annual meeting of the American Society for Clinical Investigation, May 6, 1940, at Atlantic City.

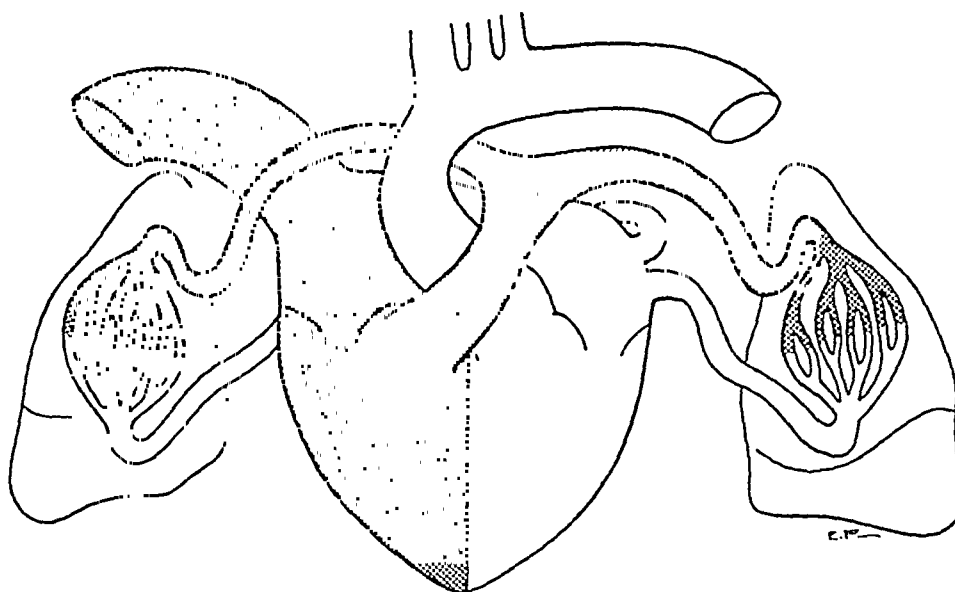


FIG. 1. A DIAGRAM OF THE NORMAL CIRCULATION

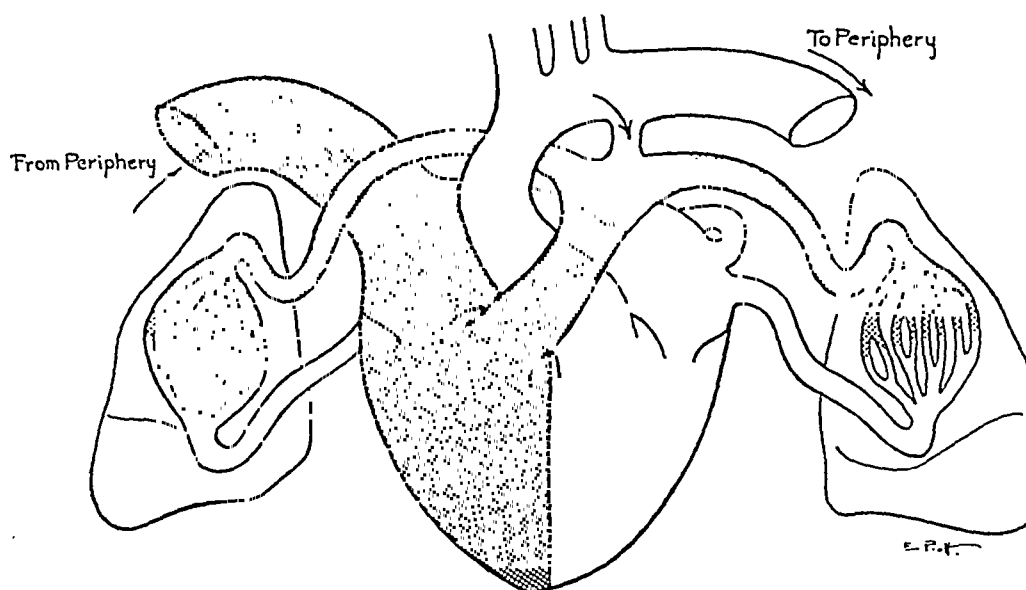


FIG. 2. A DIAGRAM OF THE CIRCULATION WHEN THE DUCTUS ARTERIOSUS IS PATENT

When a patent ductus arteriosus permits the passage of blood from the aorta to pulmonary artery, the output of the *right* ventricle is made up of blood which comes to this ventricle *from the peripheral circulation*<sup>2</sup> and *from no other source*. The volume of the peripheral flow and the output of the right ventricle are identical. The volume of the peripheral blood flow can be calculated if the oxygen consumption per minute, the oxygen content of blood in the aorta, and the oxygen content of blood in the right ventricle (mixed venous blood) are known.

<sup>2</sup> The term "peripheral circulation" includes, of course, the flow through the coronary system.

The output of the left ventricle in patients with patent ductus arteriosus is made up of two components: the blood from the right ventricle and the blood entering the pulmonary artery by way of the ductus arteriosus. These confluent streams pass through the lungs and enter the left side of the heart. Since the blood entering the lung capillaries is thus only partly blood from the right ventricle, the oxygen content of the right ventricle blood cannot be used in calculating the pulmonary blood flow. This calculation requires knowledge of the oxygen content of blood taken from the pulmonary arterial system at a point where mixture of the two streams has occurred.

If there are at hand figures expressing the oxygen consumption per minute, the oxygen content of arterial blood, the oxygen content of mixed venous (right ventricle) blood, and *the oxygen content of the mixed arterial and venous blood in the pulmonary artery*, the output of each ventricle may be calculated according to the Fick formula. Obviously, the difference between the outputs of the two ventricles is the volume of the blood which passes through the ductus.

The operations by Gross (7) presented an opportunity to obtain directly from the pulmonary artery and other vessels the crucial samples of blood<sup>3</sup> which permitted calculation of the outputs of the two ventricles. Similar studies have been made in dogs in which a connection between aorta and pulmonary artery has been established by operation. In subsequent portions of this paper the terms "pulmonary blood flow" and "peripheral blood flow" will be used. It should now be clear that these volumes are equivalent to the outputs of the left and right ventricles, respectively.

#### OBSERVATIONS ON THE FIRST PATIENT

The application of these principles to the study of the circulation in a patient may be exemplified by a brief presentation of the findings in the first case so studied.

*Case 1 (F. S.)* was a girl of six years. She appeared thin, fragile and underdeveloped for her age. The left chest was more prominent than the right and a widespread pulsation lifted the chest wall as far out as the nipple line. A precordial thrill, which was of maximum intensity in the second left interspace, could be felt throughout the cardiac cycle but was most evident in systole. In the pulmonary area, a continuous murmur was heard which had a systolic accentuation.

The diagnosis of patent ductus arteriosus was made and operation advised. (The criteria for diagnosis and for the selection of patients suitable for operative treatment have been defined by Hubbard, Emerson, and Green (10)). Operation in this patient was carried out in November 1938 under cyclopropane anesthesia. Expo-

<sup>3</sup> In all determinations of oxygen content, the manometric apparatus of Van Slyke was used and duplicate determinations were made by the same observer. All samples of blood were taken under oil with potassium oxalate as an anticoagulant. It was not necessary to modify the method on account of the anesthesia when cyclopropane (8) was used; when ether was the anesthetic, appropriate changes in analytic methods, as described by Shaw and Downing (9) were applied.

sure of the heart and great vessels revealed the presence of a patent ductus 7 mm. in diameter and 7 to 8 mm. long. The heart was beating rapidly and vigorously. A thrill was felt over the ductus and pulmonary artery. Compression of the ductus between the fingers resulted in the abolition of the thrill and visible diminution in the amplitude of the cardiac excursions. Samples of blood were obtained from the femoral artery, the pulmonary artery when the ductus was open (opposite the entrance of the ductus), and the pulmonary artery within 15 seconds of temporary closure of the ductus.<sup>4</sup> The ductus was then ligated. Some minutes later samples of blood were obtained from the femoral artery and from the pulmonary artery to determine what the situation was after the ductus had been ligated.

When these samples were analyzed it was found that the oxygen content of blood taken from the pulmonary artery (with the ductus open) approached that of femoral artery blood so closely that the question arose as to whether this sample actually represented the blood which was entering the lungs. It was considered that this pulmonary artery sample might have been taken from the stream of arterial blood entering by way of the ductus before it was thoroughly mixed with blood from the right ventricle. This possibility prompted the series of animal experiments herein described in which it was possible to make more complete observations than in patients.

#### OBSERVATIONS ON ANIMALS

What was sought in these dog experiments was to bring about a situation in which the circulation would resemble as closely as possible that of a patient with a ductus arteriosus. Accordingly, the following operation was carried out:

Under anesthesia with pentobarbital sodium or alpha chloralose, the chest was opened and the left lung partially collapsed. Respirations were maintained by posi-

<sup>4</sup> This sample requires explanation. What was sought was a sample of blood having the oxygen content of the right ventricle blood ("mixed venous blood") when the ductus was open. This could have been obtained directly, if one had been willing to puncture the right ventricle. It is technically preferable to take this sample from the pulmonary artery. This can be done, provided the ductus is temporarily clamped to prevent the entrance of blood from the aorta. Since this manipulation and sampling were completed within 15 seconds (*i.e.*, probably before recirculation could take place), the sample drawn should represent the right ventricular blood during the time when the ductus was open.



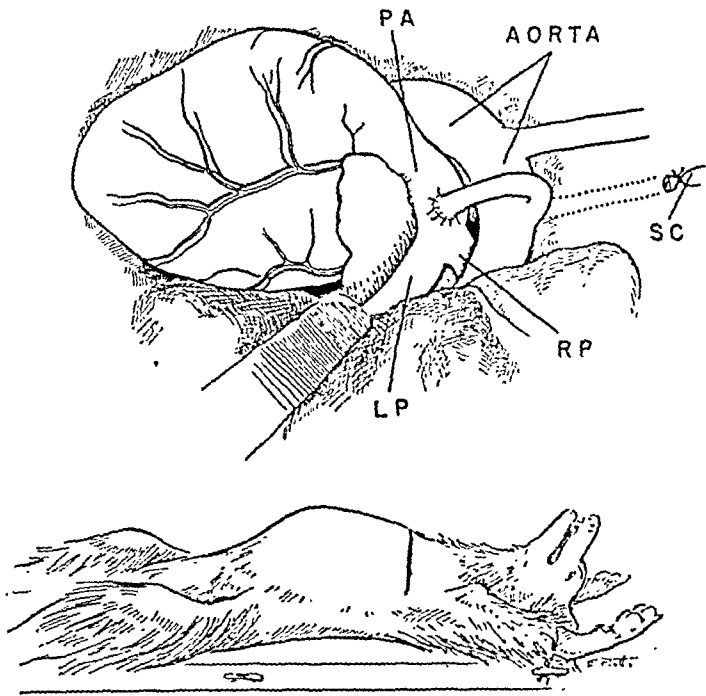


FIG. 3. METHOD EMPLOYED FOR PRODUCING AN AORTA-PULMONARY ARTERY FISTULA IN DOGS

The left subclavian artery was freed from its bed along the dotted line and then divided high in the thorax. The proximal portion of this artery was then turned downward and anastomosed to the pulmonary artery. Lower sketch shows position of the operative incision.

L P—Left pulmonary artery. R P—Right pulmonary artery. P A—Pulmonary artery. S C—Left subclavian artery.

tive pressure. Samples of blood were drawn from the pulmonary artery and the aorta. The left subclavian artery was then divided about 3 cm. above its origin from the aorta and the proximal portion sutured into the pulmonary artery just above its bifurcation (Figure 3). In this way a communication was made between the aortic arch and the pulmonary artery. When this anastomosis was completed, the action of the heart became much more vigorous and a thrill could be felt over the pulmonary artery. Samples of blood were drawn from the heart and from various points in the great vessels. Oxygen consumption was determined at the end of the experiment by the Benedict-Roth apparatus, using a Blalock mask (11). In some experiments blood volume determinations were carried out by the method of Gibson and Evans (12).

In some cases the pressures in the aorta and pulmonary artery were directly measured by connecting manometers with the needles inserted into the vessels. It was found convenient to use a mercury manometer for the determination of aortic pressure and a water manometer for that in the pulmonary artery.

Table I summarizes the results obtained in the first dog. These observations show that, after the communication is established, the pulmonary

TABLE I

Observations on the circulation of Dog number 4-39 before and after the establishment of a communication between aorta and pulmonary artery, including studies of the mixture of the confluent streams

Dog number 4-39

Source of blood	Oxygen content	Arterio-venous oxygen difference	Blood flows	Oxygen capacity
	cc. per liter	cc. per liter	liters per minute	cc. per liter
JANUARY 7, 1939*				
CIRCULATION INTACT				
Aorta.....	186.2	49.2	2.05 (Pulmonary or peripheral blood flow)	241.1
Pulmonary artery.....	137.0			
AORTA-PULMONARY ARTERY COMMUNICATION ESTABLISHED				
1. Left ventricle.....	201.6	35.7	2.83 (Pulmonary blood flow)	240.5
2. Pulmonary artery near valves	150.2			
3. Pulmonary artery opposite communication.....	171.3			
4. Left pulmonary artery at root of lung.....	165.8}	74.2	1.36 (Peripheral blood flow)	240.1
5. Right pulmonary artery at root of lung.....	165.9}			
6. Right ventricle.....	127.4	65.3	1.55 (Peripheral blood flow)	
7. Pulmonary artery immediately after temporary occlusion of communication..	136.3			

\* Basal oxygen consumption = 101 cc. per minute.

artery contains blood with a higher oxygen content than that of the blood in the right ventricle. This is evidence that blood from the aorta is entering the pulmonary artery through the artificial connection.

To determine the degree of mixture of the two streams of blood at various points, samples were taken from several parts of the pulmonary artery between the pulmonary valves and the lung roots. The oxygen content of a sample taken from the pulmonary artery near the pulmonary valves was 150.2 cc. per liter, while that of a sample taken immediately opposite the entrance of the stream from the transplanted subclavian artery was 171.3 cc. per liter. However, samples taken from the left and right branches of the pulmonary artery (several centimeters distal to the entrance of the fistula) gave values which were *essentially identical* (165.8 and 165.9 cc. per liter). These results are shown graphically in Figure 4. This agreement in oxygen content of blood going to both lungs would appear to indicate that at these points

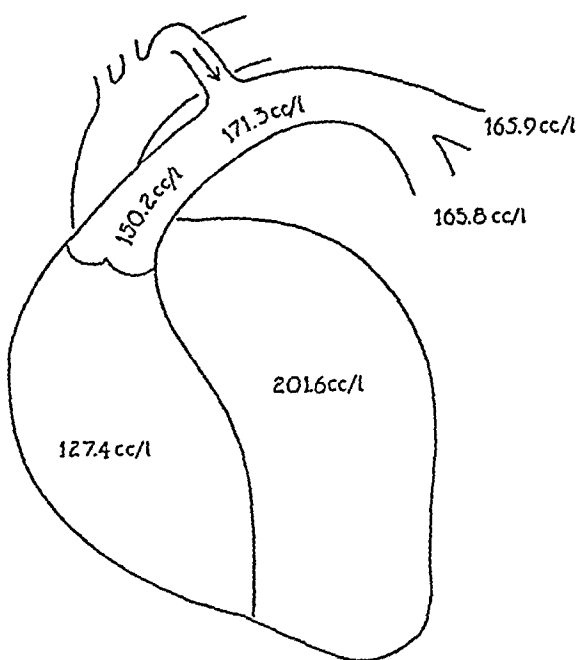


FIG. 4. THE OXYGEN CONTENT OF BLOOD IN VARIOUS PARTS OF THE HEART AND GREAT VESSELS OF DOG NUMBER 4-39 AFTER THE ESTABLISHMENT OF A COMMUNICATION BETWEEN AORTA AND PULMONARY ARTERY (SEE TABLE III)

mixture had occurred. Since this was so, *samples which are to be used in the calculation of the pulmonary blood flow in patients with patent ductus arteriosus should be taken from the pulmonary artery at a point as far as possible distal to the entrance of the ductus.*

A theoretical source of error concerns the time of the cardiac cycle during which blood samples are taken. It is probable that the flow from aorta to pulmonary artery continues through both systole and diastole, perhaps with diminishing volume in diastole. The flow from right ventricle into the pulmonary artery is limited to systole. Therefore, we might expect to find a difference between samples obtained from the pulmonary artery in systole and in diastole. However, the usual sample requires over 10 seconds to draw, and represents sampling during 15 or more complete cardiac cycles. This suggested error is probably minimal.

The calculations in Table I concerning blood flow in the dog are illuminating. In the intact animal, the output of both the right and the left ventricle was 2.05 liters per minute. With the

shunt established, the output of the left ventricle increased to 2.83 liters per minute, while that of the right, representing the flow through the periphery, fell to 1.36 liters per minute. Thus the amount flowing through the shunt is calculated to be 1.47 liters per minute, or 52 per cent of the left ventricle's output.

The experiments in the dog also present an opportunity to test the validity of the method used in obtaining "mixed venous blood" in patients when the ductus is open (*Cf.* footnote 4). Sample 7 in Table I was obtained in accordance with this method (temporary occlusion of the artificial ductus); the oxygen content of this blood was within one volume per cent of the oxygen content of blood directly aspirated from the right ventricle. A similar agreement was observed in another dog. The figures for the oxygen content of "mixed venous blood" when the ductus is open are therefore only approximate; it will be realized that these values may be slightly higher but are never lower than the true oxygen content of "mixed venous blood."

A second set of observations made on this dog after a period of 3 months, with the communication open, showed a smaller but nevertheless definite difference between the left and right ventricular outputs. Again, blood samples taken from the left and right pulmonary arteries below the bifurcation showed that essentially complete mixing of the aortic and pulmonary artery blood had occurred at these points.

A similar operation in a second, third, and fourth dog produced circulatory changes which are outlined in Tables II, III, and IV.

One other special point of technique remains to be considered. The observations just presented on dogs showed that subclavian blood entering the pulmonary artery proximal to or at its bifurcation was evenly distributed between right and left pulmonary artery branches. Occasionally, the ductus arteriosus of humans enters the *left* pulmonary artery. The opening of the ductus into the left pulmonary artery is usually close to its origin from the main trunk. According to Gérard (13), it is almost always in close relation to the bifurcation, but in one of the patients studied (Case 4) the ductus (observed at autopsy) was found to enter the left branch of the pulmonary artery 15 mm. beyond the bifurcation. It is obvious that a duc-

tus in such a position might distribute different amounts or mixtures of blood to the two lungs, and hence be a source of error if a sample is taken from only one branch of the pulmonary ar-

TABLE II

*Observations on the circulation of Dog number 17-39 after the establishment of a communication between aorta and pulmonary artery*

Dog number 17-39

Source of blood	Oxygen content	Arterio-venous oxygen difference	Blood flows	Oxygen capacity
	cc. per liter	cc. per liter	liters per minute	cc. per liter
JANUARY 27, 1939*				
1. Left ventricle.....	168.4			179.5
2. Pulmonary artery opposite communication.....	132.0			
3. Left pulmonary artery at root of lung.....	127.2	41.2	6.19 (Pulmonary blood flow)	
4. Right ventricle.....	81.0	87.4	2.92 (Peripheral blood flow)	185.6
MARCH 1, 1939†				
1. Aorta.....	143.0			
2. Left pulmonary artery at bifurcation.....	107.6	35.4	5.73 (Pulmonary blood flow)	
3. Right ventricle.....	65.6	77.4	2.62 (Peripheral blood flow)	

\* Basal oxygen consumption = 255 cc. per minute.

† Basal oxygen consumption = 203 cc. per minute.

TABLE III

*Observations on the circulation of Dog number 55-39 before and after the establishment of a communication between aorta and pulmonary artery*

Dog number 55-39

Source of blood	Oxygen content	Arterio-venous oxygen difference	Blood flows
	cc. per liter	cc. per liter	liters per minute
APRIL 12, 1939*			
Aorta.....	166.1		
Right ventricle.....	122.7	43.4	4.02 (Pulmonary or peripheral blood flow)
AORTA-PULMONARY ARTERY COMMUNICATION ESTABLISHED			
1. Aorta.....	175.4		
2. Right pulmonary artery.....	132.9		
3. Left pulmonary artery.....	136.5	40.7	4.29 (Pulmonary blood flow)
4. Right ventricle.....	94.3	81.1	2.15 (Peripheral blood flow)

\* Basal oxygen consumption = 174.5 cc. per minute.

TABLE IV

*Observations on the circulation of Dog number 63-39 before and after the establishment of a communication between aorta and pulmonary artery*

Dog number 63-39

Source of blood	Oxygen content	Arterio-venous oxygen difference	Blood flows	Oxygen capacity
	cc. per liter	cc. per liter	liters per minute	cc. per liter
JUNE 1, 1939*				
CIRCULATION INTACT				
Aorta.....	206.0			229.9
Right ventricle.....	152.1	53.9	2.31 (Pulmonary or peripheral blood flow)	
AORTA-PULMONARY ARTERY COMMUNICATION ESTABLISHED				
1. Aorta.....	229.4			252.3
2. Left pulmonary artery.....	206.6	22.8	5.47 (Pulmonary blood flow)	
3. Right ventricle.....	133.5	95.9	1.30 (Peripheral blood flow)	
JULY 26, 1939†				
1. Left ventricle.....	213.3			
2. Left pulmonary artery.....	187.4	25.9	5.23 (Pulmonary blood flow)	
3. Right ventricle.....	140.7	72.6	1.87 (Peripheral blood flow)	

\* Basal oxygen consumption = 124.8 cc. per minute.

† Basal oxygen consumption = 135.5 cc. per minute.

tery. Accordingly, an attempt was made to reproduce such a situation in an animal.

The experiment was carried out in the same manner as the dog experiments already described, except that the anastomosis was made between the subclavian artery and the *left branch* of the pulmonary artery. The point of anastomosis was 15 mm. distal to the bifurcation of the pulmonary artery. After the establishment of the connection, samples of blood were obtained from the aorta, from the right ventricle, and from several points in the pulmonary artery and its main branches. The results are set forth in Table V.

This table shows that the oxygen content of blood in the main pulmonary artery is higher than that of blood in the right ventricle; the same is true of blood in the right branch. Therefore, some arterial blood which has entered the left pulmonary artery via the anastomosis is carried back into the main pulmonary artery and so gains access to the right lung. The difference in the oxygen content of blood in the two branches, however, makes it quite clear that more of the shunted

TABLE V

*Observations on the circulation of Dog number 181-39 before and after the establishment of a communication between aorta and left branch of pulmonary artery*

Dog number 181-39

Source of blood	Oxygen content	Arterio-venous oxygen difference	Blood flows	Oxygen capacity
	cc. per liter	cc. per liter	liters per minute	cc. per liter
NOVEMBER 21, 1939*				
CIRCULATION INTACT				
Aorta.....	172.7	81.4	1.60 (Pulmonary or peripheral blood flow)	202.2
Pulmonary artery.....	91.3			
AORTA-PULMONARY ARTERY COMMUNICATION ESTABLISHED				
1. Aorta.....	178.5	30.7	4.23 (?Pulmonary blood flow)	213.9
2. Main pulmonary artery...	147.8			
3. Left pulmonary artery....	156.9	21.6	6.02 (?Pulmonary blood flow)	
4. Right pulmonary artery..	148.6	29.9	4.35 (?Pulmonary blood flow)	
Right ventricle.....	104.9	73.6	1.77 (Peripheral blood flow)	

\* Basal oxygen consumption = 129.5 cc. per minute.

blood is going to the left lung than to the right. If we could assume that equal volumes of oxygen are absorbed by the two lungs, the volume of blood flow through each lung could be calculated. In normal people, according to Björkman (14), about 53 per cent of the total oxygen is absorbed by the right lung and about 47 per cent by the left. But in patients or animals undergoing an operation of this type, the left lung is partly collapsed, and it is likely that less than its usual share of oxygen is being absorbed by it. Therefore, if the ductus enters the left branch of the pulmonary artery at a point much beyond the bifurcation, the calculations of pulmonary blood flow are not exact, although in the example cited in Table V the changes are still significant. One is not always certain, even after operative exposure, of the precise position of the opening of the ductus in relation to the bifurcation and it is possible, therefore, that in almost any given case it may open into the left branch. However, we have now made measurements in five patients and, while the ductus probably enters the pulmonary artery near the bifurcation in all of them, it is extremely improbable (13, 15) that it enters the left branch in more than the one that is known.

A few observations were made on the relative pressure in the aorta and the pulmonary artery of dogs before and after the establishment of the artificial communication. These are shown in Table VI. In two of the three animals the pressure in

TABLE VI

*Pulmonary artery pressure before and after the establishment of a communication between aorta and pulmonary artery*

Dog number	Time	Aortic pressure	Pulmonary artery pressure
		mm. of mercury	mm. of mercury
17-39	Before operation	116	16
	After operation 5 weeks later		27 22
55-39	Before operation	124 112	16 17
	After operation		
63-39	Before operation	150	16
	After operation 8 weeks later		24 19

the pulmonary artery rose. In no case did it approach the pressures in the aorta, presumably because the resistance in the lung is relatively low, as pointed out by Levy and Blalock (16).

TABLE VII

*Blood volumes in three dogs before and after establishment of a communication between aorta and pulmonary artery*

Dog number	Time	Weight	Plasma volume	Red blood cell volume	Total blood volume	Hematocrit
		kgm.	cc.	cc.	cc.	per cent
17-39	Before operation	26.1	1280	1010	2290	44.0
	5 weeks after operation	24.7	1440	770	2210	34.8
	9 weeks later		1655	915	2570	35.6
55-39	Before operation	22.2	1225	795	2020	39.3
	4 weeks after operation*	19.7	1040	770	1810	42.4
63-39	Before operation	21.2	1078	897	1975	45.4
	8 weeks after operation	22.5	1890	1990	3880	51.4

\* At this time the communication was found to be thrombosed.

Table VII summarizes studies of the blood volume in three dogs. In two dogs (number 17-39 and number 63-39) in which the evidence of the shunt persisted for 2 months or more, there was a significant increase in total blood volume, involving both plasma and cells. The dog (num-

ber 63-39) with the largest shunt (as shown by a pulmonary blood flow four times the peripheral flow) also exhibited the greatest increase in total blood volume—from 1975 cc. to 3880 cc. In one dog (number 55-39) in which the shunt closed spontaneously, this increase did not take place. In dog number 17-39 an increase in plasma volume took place in spite of an anemia due to infection, which apparently prevented an increase in cell volume.

#### CONCLUSIONS FROM FIVE DOG EXPERIMENTS

1. After the establishment of the aortic-pulmonary artery connection, the oxygen content of the blood in the pulmonary artery is always greater than that in the right ventricle. Therefore, the blood flow through the shunt is toward the pulmonary artery.

2. In two dogs, a comparison of the oxygen content of blood from the right and left branches of the pulmonary artery at the hili showed an approximate identity, indicating adequate mixture.

3. In each case, the shunt from the aorta to the pulmonary circulation was more than 50 per cent of the output of the left ventricle; the output of the left ventricle, therefore, was more than twice that of the right. In one animal (Table IV) the shunt was 75 per cent and the left ventricle was putting out four times the output of the right. The situation in this dog was essentially the same 8 weeks later.

4. In some animals, the left ventricular output was not greatly increased but the flow of blood to the periphery was diminished. In others the output of the left ventricle increased to such an extent that the volume expelled by the right was less severely reduced.

5. When the subclavian artery was anastomosed to the left branch rather than the main pulmonary artery, the arterial blood streams were not equally distributed to the right and left lungs. Under these circumstances, knowledge of the oxygen content in the left pulmonary artery did not allow the quantitative estimation of the pulmonary blood flow, although certain qualitative information was obtained.

6. In two of the three dogs in which the pulmonary blood pressure was measured before and after the subclavian artery was anastomosed with

the pulmonary artery, the pulmonary pressure rose.

7. In two animals with a large shunt a definite increase in total blood volume was observed.

#### OBSERVATIONS IN PATIENTS

In the patients to be considered now, each pulmonary artery sample was drawn from a point where, according to the animal experiments, mixture could be expected to have taken place; that is, from the left pulmonary artery as it entered the lung root. Under the conditions of this operation the right pulmonary artery is usually not accessible.

Observations were made on each of six patients subjected to ligation of a patent ductus arteriosus between November 1938 and December 1939. The data are presented in Tables VIII to XIX, inclusive, and consist of two parts: One part deals with the pulmonary and peripheral blood flow before and after ligation of the ductus and with the volume of the shunt. *The cardiac outputs were not basal. They were determined during anesthesia, and when the chest was open, and there is evidence (to be presented later) that they were much above the basal level. Therefore, these cardiac outputs cannot be compared with basal outputs in normal people or with other measurements of the circulation made in our patients under basal conditions.* The other part deals with other measurements before and after ligation, including height, weight, arterial blood pressure (basal and after exercise), venous pressure, circulation time, oxygen saturation of the arterial blood, plasma volume, red blood cell volume, vital capacity (basal and after exercise) and oxygen consumption. *Except where noted, these groups of data were obtained under standard basal conditions.* The exercise test varied somewhat with different individuals, but in general consisted of 1 to 2 minutes at 100 steps per minute over stairs. The venous pressure was measured by the direct method described by Lyons, Kennedy and Burwell (17); the circulation time was measured by sodium dehydrocholate; the blood volume by the method of Gibson and Evans (12).

#### PROTOCOLS

*Case 1 (F. S.)* has already been described. The results of the acceptable measurements of her circulation are summarized in Tables VIII and IX.

TABLE VIII

*Observations on the blood flow in Case 1 before and after ligation of the ductus arteriosus*

Case 1 (F. S.)

Source of blood	Oxygen content cc. per liter	Arterio-venous oxygen difference cc. per liter	Blood flows liters per minute	Oxygen capacity cc. per liter
NOVEMBER 9, 1935* DUCTUS ARTERIOSUS PATENT				
Femoral artery.....	163.8			169.0
Pulmonary artery after temporary occlusion of ductus.....	135.2	28.6	4.9 (Peripheral blood flow)	
DUCTUS ARTERIOSUS LIGATED				
Femoral artery.....	166.8			
Pulmonary artery.....	144.2	22.6	6.2 (Pulmonary or peripheral blood flow)	168.8

\* Basal oxygen consumption = 139 cc. per minute.

TABLE IX

*Other observations in Case 1 before and after ligation of the ductus arteriosus*

Case 1 (F. S.)

Observations	Pre-operative	Post-operative 4 months	Post-operative 14 months
Weight, kgm.....	18.32	20.25	23.45
Height, cm.....	117.0	117.0	126.1
Arterial blood pressure, basal			
Right arm, mm. Hg.....	95/50	106/80	88/60
Left arm, mm. Hg.....	98/40	100/70	92/70
Arterial blood pressure, after exercise (1½ minutes at 100 steps per minute over stairs), mm. Hg.....	110/50*	118/60	
Venous pressure, mm. H <sub>2</sub> O.....	130		
Vital capacity, cc.....	1000	1100	1250
Oxygen consumption, cc. per minute.....	146	139	
	139		
	138		

\* Sounds heard to zero.

Case 2 (M. F.), a girl of 17 years, was known to have had heart disease since early childhood. Because of dyspnea on exertion, palpitation, precordial distress and easy fatigue, her activities were seriously limited. On physical examination she appeared slender but reasonably well-developed. There were no remarkable abnormalities except for the heart. There was moderate cardiac enlargement, chiefly to the left. A vigorous systolic thrill could be felt in the pulmonary area. This thrill was accompanied by a loud continuous murmur which had a systolic accentuation. There was also a diastolic murmur at the apex which disappeared when the ductus was ligated.

On December 22, 1938, the ductus arteriosus measuring 9 mm. in diameter was ligated under cyclopropane anesthesia. Samples of blood were taken and analyzed for

oxygen content and capacity. The results of these and other measurements are collected in Tables X and XI. Six months after this ligation some degree of communication was reestablished, as judged by the reappearance of physical signs.

TABLE X

*Observations on the blood flow in Case 2 before and after ligation of the ductus arteriosus*

Case 2 (M. F.)

Source of blood	Oxygen content cc. per liter	Arterio-venous oxygen difference cc. per liter	Blood flows liters per minute	Oxygen capacity cc. per liter
DECEMBER 22, 1938* DUCTUS ARTERIOSUS PATENT				
Aorta†.....	176.9			195.8
Pulmonary artery at hilus of lung.....	169.3	7.6	25.0 (Pulmonary blood flow)	
Pulmonary artery after temporary occlusion of ductus.....	144.4	32.5	5.8 (Peripheral blood flow)	
Amount of shunt.....			19.2	
DUCTUS ARTERIOSUS LIGATED				
Aorta.....	176.9			195.8
Pulmonary artery.....	139.0	37.9	5.0 (Pulmonary or peripheral blood flow)	192.1

\* Basal oxygen consumption = 190 cc. per minute.

† This sample obtained 16 minutes after the pulmonary sample; during this time there was no essential change in the general state of the patient.

TABLE XI

*Other observations in Case 2 before and after ligation of the ductus arteriosus*

Case 2 (M. F.)

Observations	Pre-operative	Post-operative 6 weeks	Post-operative 6 months
Weight, kgm.....	42.83	42.64	46.40
Height, cm.....	153.3	153.3	153.3
Arterial blood pressure, basal			
Right arm, mm. Hg.....	122/54	104/70	114/64
Left arm, mm. Hg.....	122/54	108/72	118/70
Arterial blood pressure, after exercise (1 minute at 100 steps per minute over stairs), mm. Hg.....	154/40*	146/72	
Venous pressure, mm. H <sub>2</sub> O.....	38	62	102
Circulation time, seconds.....	21.5-23.5	12.8	19.3
Arterial oxygen content, cc. per liter.....	169.3	185.3	
Arterial oxygen capacity, cc. per liter.....	178.0	195.5	
Arterial oxygen saturation, per cent.....	95.1	94.8	
Total blood volume, cc.....	3980	3280	3720
Plasma volume, cc.....	2430	1980	2270
Red blood cell volume, cc.....	1550	1300	1450
Hematocrit, per cent.....	38.9	39.8	38.9
Vital capacity, cc.....	2500	2200	1650
			2200†
Vital capacity after exercise, cc.....	2200	2100	
Oxygen consumption, cc. per minute.....	192	185	190
	185	181	
	194	178	

\* Sounds heard to zero.

† This determination of the vital capacity was made 12 months after operation.

TABLE XII

*Observations on the blood flow in Case 3 before and after ligation of the ductus arteriosus*

Case 3 (D. M.)

Source of blood	Oxygen content	Arterio-venous oxygen difference	Blood flows	Oxygen capacity
	cc. per liter	cc. per liter	liters per minute	cc. per liter
AUGUST 1, 1939*				
DUCTUS ARTERIOSUS PATENT				
1. Aorta.....	174.1	7.4	19.5 (Pulmonary blood flow)	186.9
2. Left pulmonary artery at hilus of lung.....	166.7			
DUCTUS ARTERIOSUS LIGATED				
3. Aorta.....	173.7	22.7	6.3 (Pulmonary or peripheral blood flow)	179.3
4. Pulmonary artery.....	151.0			

\* Basal oxygen consumption = 144 cc. per minute.

TABLE XIII

*Other observations in Case 3 before and after ligation of the ductus arteriosus*

Case 3 (D. M.)

Observations	Preoperative	Post-operative 5 months
Weight, kgm.....	25.88	30.00
Height, cm.....	137.5	140.9
Arterial blood pressure, basal		
Right arm, mm. Hg.....	70/40	90/66
Left arm, mm. Hg.....	92/48	88/56
Arterial blood pressure, after exercise (1 minute at 100 steps per minute over stairs), mm. Hg.....	108/40*	
Venous pressure, mm. H <sub>2</sub> O.....	unsatisfactory	
Circulation time, seconds.....	17.6-19.8	
Total blood volume, cc.....	2580	1825
Plasma volume, cc.....	1655	
Red blood cell volume, cc.....	925	
Hematocrit, per cent.....	35.9	
Vital capacity, cc.....	1600	
Vital capacity after exercise, cc.....	1750	
Oxygen consumption, cc. per minute.....	147	
	141	

\* Sounds not heard to zero.

Case 3 (D. M.) was a delicate appearing, thin, undernourished girl of 11 years. She had led an unusually active life rather than one of limited activity. The heart was moderately enlarged to the left. A vigorous thrill of maximum intensity in diastole was felt in the second interspace. In this same area a loud, continuous murmur was heard. The physical examination was not otherwise remarkable.

On August 1, 1939, she was operated upon under cyclopropane anesthesia and the patent ductus arteriosus measuring 8 mm. in diameter was ligated. Unfortu-

nately, since a sample of mixed venous blood was not obtained, an estimation of the flow of blood through the periphery with the ductus open could not be made. The results of the studies are collected in Tables XII and XIII.

Case 4 (L. B.) was a girl of 15 years. For many years because of dyspnea she had been unable to run and play as strenuously as her friends. She was slender, small-boned, and short. Her fingers were long and hyper-extensible. The thorax was long, the respiratory excursions appeared limited and there was some precordial fullness. Vigorous pulsations could be seen in the suprasternal notch and in both supraclavicular spaces, especially on the right. The heart was moderately enlarged to the left and its action unusually forceful. In the second left interspace a thrill was felt, most marked in systole, but at times palpable throughout the cardiac cycle. There was a systolic murmur at the apex and a loud continuous murmur in the second left interspace. The peripheral arteries showed a collapsing type of pulsation and in the femoral region there was a loud pistol shot sound.

On October 22, 1939, she was operated upon under ether anesthesia and a large patent ductus measuring 8 mm. in diameter was ligated. The results of the studies of this patient are summarized in Tables XIV and XV. This patient died 9 days after operation and at post-mortem examination was found to have an extensive mediastinitis, pericarditis and left empyema. There had been erosion of the ligatures through the ductus and extensive hemorrhage into the pleural cavity. Staphylo-

TABLE XIV

*Observations on the blood flow in Case 4 before and after ligation of the ductus arteriosus*

Case 4 (L. B.)

Source of blood	Oxygen content	Arterio-venous oxygen difference	Blood flows
	cc. per liter	cc. per liter	liters per minute
OCTOBER 22, 1939*			
DUCTUS ARTERIOSUS PATENT			
1. Aorta.....	173.7	17.7	10.3 (Pulmonary blood flow)
2. Left pulmonary artery.....	156.0		
3. Pulmonary artery after temporary occlusion of ductus.....	120.5	53.2	3.4 (Peripheral blood flow)
Amount of shunt.....			6.9
DUCTUS ARTERIOSUS LIGATED			
4. Aorta.....	157.3	45.7	4.0 (Pulmonary or peripheral blood flow)
5. Pulmonary artery.....	111.6		

\* Oxygen consumption = 183 cc. per minute.

TABLE XV

*Other observations in Case 4 before ligation of the ductus arteriosus*

(This patient died 9 days after the operation.)

Case 4 (L. B.)

Observations	Preoperative
Weight, <i>kgm.</i> .....	32.28
Height, <i>cm.</i> .....	147.1
Arterial blood pressure, basal	
Right arm, <i>mm. Hg.</i> .....	120/48
Left arm, <i>mm. Hg.</i> .....	132/30
Arterial blood pressure, after exercise (1½ minutes at 50 steps per minute over stairs), <i>mm. Hg.</i> .....	170/20*
Venous pressure, <i>mm. H<sub>2</sub>O.</i> .....	38
Circulation time, <i>seconds.</i> .....	19
Arterial oxygen content, <i>cc. per liter.</i> .....	172.4
Arterial oxygen capacity, <i>cc. per liter.</i> .....	182.9
Arterial oxygen saturation, <i>per cent.</i> .....	94.2
Total blood volume, <i>cc.</i> .....	2870
Plasma volume, <i>cc.</i> .....	1650
Red blood cell volume, <i>cc.</i> .....	1220
Hematocrit, <i>per cent.</i> .....	42.7
Vital capacity, <i>cc.</i> .....	1300
Vital capacity after exercise, <i>cc.</i> .....	1300
Oxygen consumption, <i>cc. per minute.</i> .....	164
	170

\* Sounds heard to zero.

*coccus aureus* was cultured from the blood before and after death.

Case 5 (D. S.) was a small but normally developed girl of 6 years. There were no symptoms of cardiac disability. Her physical examination was not remarkable except for the usual continuous murmur and thrill in the second left interspace and a soft systolic murmur at the apex.

On December 18, 1939, she was operated upon under cyclopropane. A patent ductus arteriosus 7 mm. in diameter was ligated. The results of the studies of this patient are presented in Tables XVI and XVII.

Case 6 (M. S.) was a young woman of 26 years of somewhat limited mental capacity. She had always had dyspnea and easy fatigue on exertion and was unable to lie flat without respiratory discomfort. She appeared ruddy and healthy. Both little fingers were deformed; the right showed contracture and the left lacked a terminal phalanx. The heart was enlarged to the left. There was an apical systolic murmur and a continuous murmur in the second left interspace which was accompanied by a thrill. The pulmonary second sound was accentuated. In the supine position, the facies became suffused but no remarkable distention of the jugular veins occurred.

She was operated upon on December 22, 1939, under ether anesthesia. The ductus measuring 8 mm. in diameter was ligated. The results of studies of this patient are set forth in Tables XVIII and XIX.

## DISCUSSION OF OBSERVATIONS ON PATIENTS

In these six patients the diagnosis of patency of the ductus arteriosus was confirmed at the operation. In each the patent ductus arteriosus was the only demonstrable anomaly. It is to be emphasized that this is a group of patients selected for operation on the basis of impressive signs or symptoms. Therefore, these ducti (which measured from 7 to 9 mm. in diameter) were presumably larger than many that are recognized.

The discussion of the observations on these six patients will, for convenience, be taken up in two

TABLE XVI

*Observations on the blood flow in Case 5 before and after ligation of the ductus arteriosus*

Case 5 (D. S.)

Source of blood	Oxygen content	Arterio-venous oxygen difference	Blood flows	Oxygen capacity
	<i>cc. per liter</i>	<i>cc. per liter</i>	<i>liters per minute</i>	<i>cc. per liter</i>

DECEMBER 18, 1939\*  
DUCTUS ARTERIOSUS PATENT

1. Aorta.....	157.9			171.6
2. Left pulmonary artery at hilus of lung.....	145.6	12.3	8.7 (Pulmonary blood flow)	
3. Pulmonary artery after temporary occlusion of ductus.....	135.6	22.3	4.8 (Peripheral blood flow)	
Amount of shunt.....			3.9	

DUCTUS ARTERIOSUS LIGATED

4. Aorta.....	159.8			170.9
5. Pulmonary artery.....	127.6	32.2	3.3 (Pulmonary or peripheral blood flow)	

\* Basal oxygen consumption = 107 cc. per minute.

TABLE XVII

*Other observations in Case 5 before ligation of the ductus arteriosus*

(Postoperative observations were not possible.)

Case 5 (D. S.)

Observations	Preoperative
Weight, <i>kgm.</i> .....	15.55
Height, <i>cm.</i> .....	101.5
Arterial blood pressure, basal	
Right arm, <i>mm. Hg.</i> .....	98/40
Left arm, <i>mm. Hg.</i> .....	114/58
Oxygen consumption, <i>cc. per minute.</i> .....	107



TABLE XVIII

*Observations on the blood flow in Case 6 before and after ligation of the ductus arteriosus*

Case 6 (M. S.)

Source of blood	Oxygen content	Arterio-venous oxygen difference	Blood flows	Oxygen capacity
	cc. per liter	cc. per liter	liters per minute	cc. per liter
DECEMBER 22, 1939*				
DUCTUS ARTERIOSUS PATENT				
1. Aorta.....	174.8	10.8	14.1 (Pulmonary blood flow)	177.0
2. Left pulmonary artery.....	164.0			
3. Pulmonary artery after temporary occlusion of ductus..	149.3	25.5	6.0 (Peripheral blood flow)	
Amount of shunt.....			8.1	
DUCTUS ARTERIOSUS LIGATED				
4. Aorta.....	173.7	24.3	6.3 (Pulmonary or peripheral blood flow)	
5. Pulmonary artery.....	149.4			

\* Oxygen consumption = 153 cc. per minute.

TABLE XIX

*Other observations in Case 6 before and after ligation of the ductus arteriosus*

Case 6 (M. S.)

Observations	Preoperative	Post-operative 1 month
Weight, kgm.....	52.20	54.45
Height, cm.....	154.6	
Arterial blood pressure, basal		
Right arm, mm. Hg.....	128/66	115/80
Left arm, mm. Hg.....	118/62	
Arterial blood pressure, after exercise.....	unsatisfactory	
Venous pressure, mm. H <sub>2</sub> O.....	101	82
Circulation time, seconds.....	13.8-15.0	11.6
Arterial oxygen content, cc. per liter.....	161.4	
Arterial oxygen capacity, cc. per liter.....	171.2	
Arterial oxygen saturation, per cent.....	94.2	
Total blood volume, cc.....	4130	3720
Plasma volume, cc.....	2520	2260
Red blood cell volume, cc.....	1610	1460
Hematocrit, per cent.....	38.9	39.0
Vital capacity, cc.....	2700	2300

parts: The first part (A) deals with the direction and volume of the flow through the ductus and with the output of the two ventricles. The second part (B) deals with a variety of other measurements made before and after ligation of the ductus.

#### *A. Observations concerning blood flow*

The patients were not in a basal state when the observations on pulmonary and peripheral blood

flow were made. They were anesthetized, the chest was opened, and the respiration carried on under positive pressure. It is known that the output of the heart may be altered by various anesthetic agents. Regarding ether, Blalock (18) has studied the problem in dogs by one method and Snyder (19) in humans by another. It appears that at moderate levels of surgical anesthesia, there is an increase in the cardiac output while, at deeper levels an actual decrease may occur. Robbins and Baxter (20) have demonstrated that when cyclopropane is used, the cardiac output is increased as much as 45 to 50 per cent under conditions of surgical anesthesia. These increases are presumably related to dilatation of the peripheral vascular bed and are not due to a large increase in the oxygen consumption. In two cases in which the oxygen consumption during ether anesthesia was determined at the time of operation, it was not significantly different from that observed under standard conditions. When cyclopropane was used, a determination of the oxygen consumption under anesthesia was not made. However, it was shown by Robbins and Baxter (20) that, in dogs, the amount of oxygen used under basal conditions and under cyclopropane anesthesia does not differ by a significant amount.

The anesthetic agents used in the dog experiments, according to the available information (21), do not increase the cardiac output and may decrease it. This difference is to be kept in mind when comparing the observations in dogs with those in patients.

The analyses of blood from the aorta and the pulmonary artery in these patients offer the first direct demonstration of the direction of the flow of blood through the patent ductus, a point hitherto the subject of many assumptions. In these patients the blood in the pulmonary artery contains more oxygen than the blood in the right ventricle; therefore, arterial blood must enter the pulmonary artery by way of the ductus. Moreover, the oxygen saturation of femoral artery blood (shown in Tables X, XIV and XVIII to be from 94.2 to 95.1 per cent) indicates that there is no significant flow of venous blood in the reverse direction. This, of course, explains the absence of cyanosis

in patients whose only lesion is a patent ductus arteriosus.<sup>5</sup>

Are there any circumstances under which the flow will be from pulmonary artery to aorta? The obvious answer is that such flow will occur when the pressure in the pulmonary artery exceeds that in the aorta. The resistance necessary for the development of such an elevated pulmonary pressure will usually be back pressure from a failing left ventricle, or may occasionally be some form of pulmonary vascular disease. Obviously, this resistance must be *distal* to the origin of the ductus if it is to cause a flow of mixed venous blood through it.<sup>6</sup>

TABLE XX

*The blood flows of six patients before and after the ligation of the patent ductus arteriosus*

(The blank spaces indicate that certain samples of blood either were not obtained or were technically not acceptable.)

Patient number	Blood flow before ligation		Volume of shunt	Percentage of left ventricular output shunted through ductus	Peripheral and pulmonary blood flow after ligation
	Peripheral	Pulmonary			
	liters per minute	liters per minute	liters per minute		liters per minute
1	4.9				6.2
2	5.9	25.4	19.5	77	5.1
3		19.1			6.2
4	3.4				4.0
5	4.8	8.7	3.9	45	3.3
6	6.0	14.1	8.1	57	6.3

The volume of the flow is quite as interesting as the direction and much more unexpected. Table XX summarizes the essential measure-

<sup>5</sup> A contrary view has been expressed by Koza and Mělnka (22) who found an arterial saturation of 84 per cent in one patient who was said to exhibit the classical signs of patent ductus arteriosus.

<sup>6</sup> Holman (23) has assumed that the propulsive force exerted by a hypertrophied right ventricle can produce a blood pressure in the pulmonary artery greater than the pressure produced in the aorta by a less well-developed left ventricle. The experiments of Levy and Blalock (16), and those reported in the early part of this paper, show that even when a powerful left ventricle is pumping large volumes of blood into the pulmonary artery, the pressure in the pulmonary artery does not rise to anything like that in the aorta. The obvious explanation is that the resistance in the lung is much less than in the periphery.

ments.<sup>7</sup> In the patients studied, from 4 to 19 liters of blood per minute are found to be flowing

<sup>7</sup> Certain possibilities of error exist which may affect the results in any given case. Ideally, blood samples to be used in the calculation of cardiac output should be drawn simultaneously. Under the conditions of operation this is not practicable, but with one exception (Table X) the samples in each patient were obtained within a few minutes of one another. The errors attributable to inadequate mixing of the two currents of blood in the pulmonary artery are presumably met by taking this sample from near the pulmonary hilus where our observations on animals indicate that mixture has occurred.

In the observations on humans, "mixed venous blood," when the ductus was open, was obtained by a method already described.<sup>4</sup> This method, while not absolute, has been shown in the dog experiments to offer an acceptable approximation.

There is also the occasional occurrence of a ductus which enters the left branch of the pulmonary artery, a point already considered in the observations on animals. These various sources of error have been recognized and attempts have been made to eliminate them. It is believed that the general order of figures is essentially correct. This conclusion is borne out by the similarity in the measurements in humans and dogs and by the impressive diminution in the cardiac excursion with occlusion of the ductus, as shown both by direct observation at the operating table and by comparison of x-ray kymograms made before and after operation.

In Case 2 the pulmonary flow of 25.4 liters per minute approaches the volume considered by Bainbridge (24) to represent the upper limit of cardiac capacity. It should be noted that this volume was observed during operation and that this patient exhibited the most severe symptoms—the largest heart, the greatest amplitude of cardiac excursion and the largest ductus.

Another point already obvious to those who have worked with cardiac output is that the smaller the arteriovenous difference the greater the effect of analytical errors. Thus, if the oxygen consumption is 200 cc. per minute, and the arteriovenous oxygen difference is 50 cc. per liter, the cardiac output will be 4 liters per minute. If the arteriovenous difference changes from 50 cc. to 52 cc., the calculated cardiac output will become 3.8 liters, an inconsiderable change. However, if the oxygen consumption is 200 cc. and the arteriovenous difference is 10 cc. per liter, the calculated output will be 20 liters per minute. At this level if the arteriovenous difference changes from 10 to 12, i.e. by 2 cc. per liter, the calculated output will become 16.6 liters, a major change. Thus, when large outputs are calculated, such as those which are reported in Case 2, they are bound to have a greater error than when the arteriovenous difference is larger. This criticism does not affect the general significance of our results, but does mean that figures at this level of output are less precise than those obtained at lower levels.

from the high pressure area in the aorta to the relatively low pressure area in the pulmonary artery. These volumes constitute 45 to 75 per cent of all the blood entering the aorta from the left ventricle. This arterial blood flows through the ductus to the pulmonary artery and via the lungs back to the left ventricle without having passed through the peripheral circulation and through the right ventricle.

The cardiac output is increased by the circumstances under which the observations were made. This is shown by the fact that the cardiac output, after ligation of the ductus (Table XX), was above the basal level in every patient. In several patients it was as much as 100 per cent above the calculated basal values. The figures for the output were obtained by the reliable direct Fick method immediately after ligation without the complications of mixing and timing which exist when the ductus is open. These are the figures which are to be compared with the figures for pulmonary and peripheral blood flow when the ductus is open.

The total flow through the lungs in these patients was from 10 to 25 liters per minute. This flow represents the output of the right ventricle plus that part of the output of the left which is shunted through the ductus. Since the lungs, and consequently the left ventricle, have these two sources of blood, and the right ventricle only one (*i.e.*, the periphery), there is a difference in the output of the two ventricles. These inequalities in their output must affect the work performed by the two ventricles. In the patients studied, the left ventricle was putting out at the time of the observations from two to four times the volume expelled by the right.

The work of the right ventricle is probably not greatly changed, since it is certainly not pumping more than the usual amount of blood and may often be pumping less. The pulmonary pressure against which this blood is pumped may be elevated to some degree in the presence of a patent ductus, but probably when the lungs are normal this increase is not great.

These speculations are in accord with the observation that right ventricular preponderance is seldom found after the age of infancy when a patent ductus arteriosus is the only congenital cardiac abnormality.

The marked increase in the output of the left ventricle implies an increase in work unless there is a corresponding diminution in the mean aortic blood pressure. This mean pressure is somewhat lowered in these patients but not sufficiently to prevent a considerable increase in the work of the ventricle. This work may be further increased by still another factor; that is, the velocity imparted to the blood. This factor becomes increasingly important when dealing with such a large volume per minute as is put out by the left ventricle in these patients. Since these data are not at hand, it is not possible to make precise calculations of the work of the left ventricle. However, it is probably justifiable to conclude that the work of the left ventricle is considerably increased. This suggestion is borne out by the observation that, when heart failure occurs in these patients, it is usually predominantly left-sided failure, and by the fact that the x-ray film commonly shows a dilatation or hypertrophy of the left ventricle. Indeed, it is surprising that preponderance of the left ventricle is not more common in the electrocardiographic tracing than the observations of Schnitker (25) and ourselves have shown it to be. It appears that in these patients the response of the left ventricle to the demand for increased work is like the response in patients with arteriovenous fistula; that is, it is mainly a reversible dilatation and only ultimately does hypertrophy occur.

The observations on blood flow throw light on the problem of the adjustment of the circulation to the abnormal situation created by the patency of the ductus arteriosus. Theoretically, the diversion of a considerable volume of blood per minute from the aorta to the pulmonary artery may result in: (1) a diminution in the blood supply to the periphery, (2) an increase in the output of the left ventricle, or (3) a combination of the two.

Actually, in every patient and in every dog in which studies of the blood flow were made there was an increase in the output of the left ventricle. This increase, even in the presence of a large leak, was in some patients sufficient to maintain the peripheral flow at or near its normal level. In other patients, however, this compensation was not complete. The evidence for this is that the

peripheral flow increased in two patients when the ductus was ligated.

Examples of both types of adjustment are shown by the measurements of the blood flow in dog number 63-39 (Table IV). After the establishment of the artificial communication, the output of the left ventricle rose and the flow to the periphery fell. After 7 weeks during which there

tors, including the size of the ductus. In the patients reported, the ductus was 7 mm. or more in diameter and the changes in the circulation were severe. When the ductus is smaller, the changes are probably less severe and the required adjustments less extensive. Other factors as yet unknown may also affect the adjustment of the circulation to this lesion.

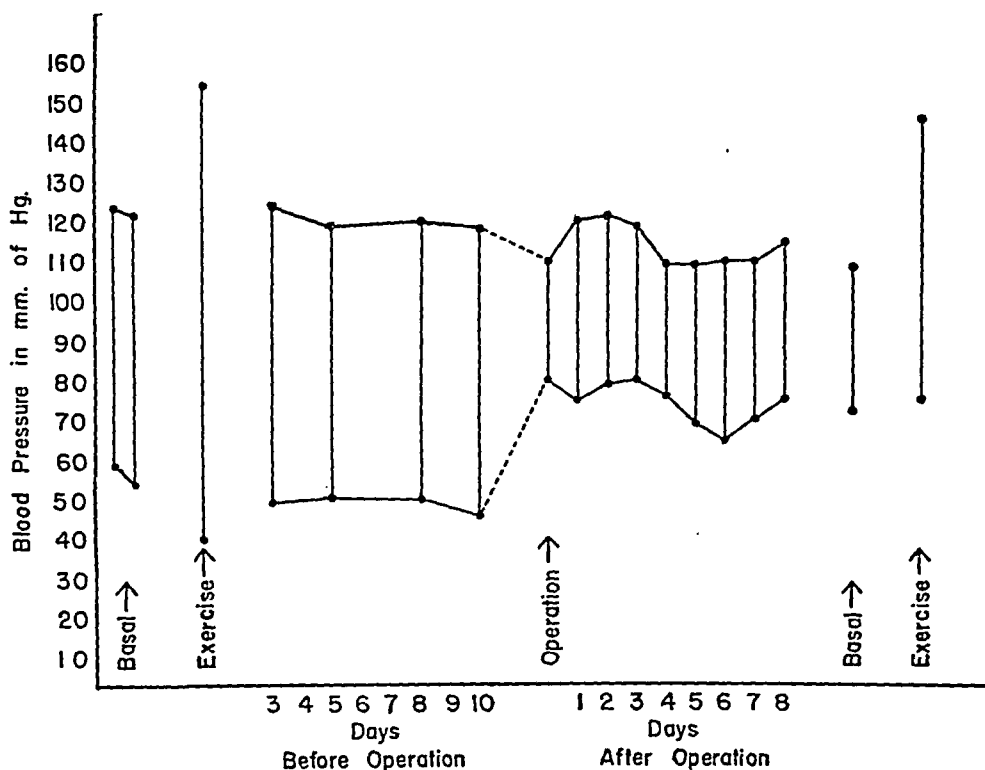


FIG. 5. ARTERIAL BLOOD PRESSURES IN CASE 2 UNDER VARYING CIRCUMSTANCES BEFORE AND AFTER LIGATION OF THE DUCTUS ARTERIOSUS

was an increase in the total blood volume, the output of the left ventricle was still 5.2 liters and the flow to the periphery had risen from 1.3 to 1.9; that is, it approached the control value of 2.3.

Thus, the patient with a large patent ductus arteriosus must either suffer a decrease in the blood supplied to the organs by the peripheral circulation or there must be extra work on the part of the left ventricle. This increase in work must limit the cardiac reserve and in the long run may be a factor in the onset of failure of the left ventricle.

The degree and kind of compensatory adjustment may be presumed to vary with many fac-

### B. Other observations

*Arterial blood pressure.* Studies of the blood pressure in these six patients (some of whom have been reported by Gross and Hubbard (1) and by Gross (26)) show a low diastolic level and a wide pulse pressure (determined according to the standard procedure defined by the American Heart Association) as constant findings. This is in accordance with the observations of Bohn (27). The level of the diastolic pressure is comparable to that seen in patients in whom there is a leakage of blood from the arterial system by way of an arteriovenous fistula. In our six patients the initial low diastolic level was found to be still lower

after mild exercise. The exercise consisted of walking over steps at a rate of about 100 steps a minute for 1 minute, a degree of exertion which in normal people produces no change or even a slight rise in the diastolic pressure. Not only was there a fall in the diastolic pressure but also the arterial sounds were often heard to zero. Ligation of the ductus was followed by immediate and striking rise in the diastolic blood pressure. The changes are illustrated in Figure 5.

*Venous blood pressure.* This measurement by the direct method was made before and after operation in a few patients. No constant changes were observed and all the readings were within limits accepted as normal.

*Circulation time.* Before operation the circulation time was from 14 to 23.5 seconds. That is somewhat longer than the expected time in children. In the two cases in whom satisfactory measurements were made after operation it was shorter than before operation. In Case 2, in whom there was a partial recurrence of the leak, the circulation time was 21 to 23 seconds before operation, 13 seconds 6 weeks after operation, and 19 seconds 5 months later when recurrence had taken place. Since pulmonary congestion (increase in the amount of blood in the lungs) is of constant occurrence in these patients, it is probable that these suggestive changes in circulation time are to be explained on this basis.

*Total blood volume.* This measurement was made with the generous collaboration of Dr. Gibson. Satisfactory observations before and after ligation were obtained in two patients and in both of them the hematocrit reading remained essentially constant. In Case 2, the total blood volume was 3980 cc. before ligation, 3280 cc. 6 weeks after operation and 3720 cc. 6 months after operation when some recurrence of the leak had taken place. In Case 6 the total blood volume was 4130 cc. during the control period and 3720 cc. 1 month after ligation of the ductus. These observations are in accord with the rise in the total blood volume which was observed in dogs after establishment of an aortic-pulmonary artery fistula. The two patients in whom these changes in total blood volume were observed had symptoms which limited their activity and were considered to be in mild congestive heart failure.

*Vital capacity.* There were no constant changes

in this measurement, in spite of an impressive diminution in the x-ray signs of pulmonary congestion following ligation of the ductus. The absence of significant change may be related to the fact that many of the patients were children and all of them had some difficulty in vigorous chest movement for a considerable period after operation.

*Nutrition and growth.* The relation of the ligation of the ductus to the state of nutrition and to rate of growth has been discussed by Gross (26). For the sake of completeness the height and weight figures are included in our tables. These figures indicate that ligation of the ductus may be followed by accelerated growth and improved nutrition. These gains suggest that abolition of the shunt has permitted a larger amount of blood to be distributed to the periphery. Such enhanced peripheral flow was actually demonstrated in two patients.

In general, the circulatory measurements that have been made in patients emphasize the extent of circulatory changes and the severity of the burden imposed on the heart when the ductus is large. They support the viewpoint that ligation of the ductus may be indicated for the purpose of relieving the heart of an eventually disabling burden and they explain quite clearly the benefit which follows operation in patients with disability from diminished reserve or congestion.

#### SUMMARY

Studies of the circulation made on six patients before and after surgical closure of an uncomplicated patent ductus arteriosus show that:

1. When the ductus arteriosus is open the blood flow is from the aorta to the pulmonary artery.
2. There is no flow of blood from pulmonary artery to aorta. Therefore, these patients do not have arterial unsaturation and are not cyanotic.
3. The volume of blood flowing from aorta to pulmonary artery varied from 4 to 19 liters per minute, which is 45 to 75 per cent of all the blood pumped into the aorta by the left ventricle. These flows occurred in patients with large ducti and under temporary conditions which are known to elevate the output of the heart.
4. The left ventricle expelled from two to four times the volume of blood expelled by the right ventricle in a given period of time.

5. Adjustment of the circulation to the patent ductus may be made by an increase in the output of the left ventricle. If this is not sufficient to compensate completely for the leak through the ductus, there may be, in addition, a diminution in the blood flow to the periphery.

Comparable studies in dogs with an artificial aorta-pulmonary artery fistula showed similar circulatory adjustments.

Knowledge of the circulatory changes which occur with patency of the ductus permits a better understanding of the signs and symptoms associated with this condition. Furthermore, these studies of the circulation supply direct evidence of the beneficial effects of operative closure of the ductus in improving the peripheral circulation in some of the patients and in reducing the work of the heart in all of them.

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# THE CARDIAC OUTPUT AND OTHER MEASUREMENTS OF THE CIRCULATION IN COARCTATION OF THE AORTA<sup>1</sup>

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The clinical diagnosis of coarctation of the aorta has been made during life with increasing frequency in the past few years. This defect has taken on increased significance recently due to attempts to explain the hypertension associated with it on the basis of renal ischemia of the Goldblatt pattern. The effect of this congenital defect on the circulation has been studied, however, in only a few patients. Blumgart, Lawrence, and Ernstene (1) reported detailed observations on two patients. The difference between the oxygen contents of the arterial and venous blood of both the arms and the legs was within normal limits. There was delay in the femoral pulse and rounding of the pulse wave. Measurement of the velocity of blood flow gave evidence of slowing of the arterial blood flow in the lower extremities.

Measurements of cardiac output are available in only four cases. Grollman and Ferrigan (2) found a normal arteriovenous oxygen difference, an increased oxygen consumption, and elevated cardiac output in one case.<sup>2</sup> Strayhorn (3), who made observations of a pregnant woman,<sup>3</sup> found the arteriovenous oxygen difference normal and the oxygen consumption and cardiac output increased. Following delivery, the oxygen consumption, cardiac output, and arteriovenous oxygen difference were all normal. Stewart and others (4) studied the dynamics of the circulation in one case.<sup>4</sup> Lequime (5) found a normal arteriovenous oxygen difference and basal metabolic rate, and a cardiac output of 3.44 liters per minute in one case.

Our studies were made on fourteen patients suffering from coarctation of the aorta who were seen in the New York Hospital during the last seven years.<sup>5</sup> In thirteen the diagnosis was made during life; in one the diagnosis was revealed at autopsy; in four of the thirteen cases the diagnosis was confirmed at autopsy. All subjects showed normal sinus mechanism. Clinical data on these fourteen cases are presented in Table I. On physical examination there were no clinical evidences of other cardiac anomalies which might, through recirculation of blood, give rise to erroneous results in the measurements.

## METHODS

All observations were made in the morning under basal conditions. Measurements of the cardiac output were made by the acetylene method, three samples of gas being taken as first recommended by Grollman (6), and as further elaborated by Grollman and others (7). Our method and the order of the procedures are exactly as we have already described (8). The following data were also collected: Oxygen consumption, vital capacity, height and weight, electrocardiograms with IVF chest leads (9), venous pressure (8, 10), blood pressures in both arms and both legs, two-meter x-ray photographs of the heart for measurement of the cardiac area (11), and cardiac volume (12).

The arm-to-tongue circulation time was estimated by the use of Decholin (8, 13). In certain patients (Cases 2, 5, 7, 8, and 9), Mascosol<sup>6</sup> (14) was used. In them the time was measured from the beginning of the injection until the patient perceived a flash of heat in the throat, perineum, hands, and feet. The inhalation of CO<sub>2</sub> was used to estimate circulation time from the lungs to the respiratory center in seven patients (15) (Table II).

<sup>1</sup> Read by title before the Fifty-Fifth Annual Meeting of the Association of American Physicians, held in Atlantic City, New Jersey, May 8, 1940.

<sup>2</sup> This patient was later seen in New York Hospital and is Case 4 in our series.

<sup>3</sup> This patient was seen in New York Hospital and is Case 14 in our series.

<sup>4</sup> This patient was seen in New York Hospital and is Case 4 in this paper.

<sup>5</sup> We are indebted to Dr. J. M. Steele for referring Cases 2 and 5 to us. They are Cases A. C. and W. G. in his paper (22).

<sup>6</sup> Mascosol, a solution described by Spier, Wright, and Saylor (14) containing magnesium sulphate, calcium gluconate, and sodium chloride, was supplied to us for these studies by The Nepera Chemical Co., Inc., of Yonkers, N. Y.



TABLE I  
Clinical data from fourteen cases of coarctation of the aorta

Case number Age Sex	Duration of symptoms	Age when diagnosis was made	Symptoms						Blood pressure				Palpable arterial pulsations in legs			Evidence of col- lateral circulation			Cardiac enlargement from cardiothoracic ratio	Aortic insufficiency	Systolic murmur	Electrocardiographic findings	Autopsy confirmation of diagnosis
			Dyspnea	Edema	Fatigue	Palpitation	Preordial pain	Claudications	Headache	Right arm	Left arm	Right leg	Left leg	Remoral	Popliteal	Dorsalis pedis	Intercostal pulsations	Bruit					
1 J. G. 15 years ♂	3 weeks	15 years	0	0	0	+	0	0	170/90 130/70	160/87 128/70	100/80 90/80	95/90 85/75	small	absent	absent	+	+	+	0	0	+	LAD*	
2 A. C. 17 years ♂	5 years	17	+	0	0	+	0	0	160/110	160/98	105/90	110/95	small	absent	absent	+	+	+	0	0	+	LAD	
3 E. B.† 28 years ♀	23 years	28	+	0	+	0	0	0	136/70	134/72	120/90	115/90	present	present	present	0	0	0	0	+	+	LAD	
4 J. F. 26 years ♂	0	23	0	0	0	0	0	0	174/60	204/60	98/82	96/84				+	+	+	+	+	+	myocardial damage	+
5 W. G. 31 years ♂	21 years	31	+	0	+	+	0	+	180/110	180/110	105/100	108/100	small	absent	absent	+	+	+	0	0	+	LAD	
6 E. S. 29 years ♀	20 years	9	+	0	+	+	0	0	200/120	195/120	125/105	150/145				+	+	+	0	0	+	LAD	
7 A. C.† 38 years ♀	3 years	38	0	0	0	+	+	0	165/110	180/110	not obtainable	not obtainable	small	absent	absent	0	0	0	0	0	+	LAD and myo- cardial damage	
8 H. K. 64 years ♂	1 year	64	+	0	+	0	0	0	200/100	190/90	not obtainable	not obtainable	absent	absent	absent	+	0	+	0	0	0	LAD and myo- cardial damage	+
9 J. L. 23 years ♂	3 years	22	+	0	+	+	+	0	160/86	160/80	110/80	108/80	small	absent	absent	+	+	+	0	0	+	myocardial damage	
10 R. Z. 27 years ♀	0	27	0	0	0	0	0	0	200/100	230/130	125/110	125/110	absent	absent	absent	+	+	+	+	0	0	LAD	
11 I. R. 48 years ♀	6 years	at autopsy	+	+	+	0	+	0	255/160	240/160		290/200	present	present	present	0	0	0	+	+	+	LAD and myo- cardial damage	+
12 G. C. 18 years ♂	6 weeks	18	0	0	0	0	0	0	168/90	138/80	not obtainable	not obtainable	absent	absent	absent	+	0	+	+	0	+	myocardial damage	+
13 W. L. 26 years ♂	3 years	24	+	+	+	0	0	0	220/120	180/120	120/98	108/100	small	small	small	+	0	+	+	+	+	LAD and myo- cardial damage	+
14 R. P. 26 years ♀	6 years	26	+	0	+	+	0	0	170/70	170/70	not obtainable	not obtainable	absent	absent	absent	+	+	+	+	0	+	LAD	

\* LAD = Left axis deviation.

† The diagnosis of coarctation of aorta was made certain either by scalloping of the ribs, collateral circulation or autopsy in all cases except 3 and 7. Case 3 was a young individual with differences in blood pressure of the arms and legs. When pregnant she was observed frequently and carefully before and after normal spontaneous labor. She developed signs of aortic insufficiency after labor which she did not have beforehand. This was interpreted as due to damage to the aortic ring or an aortic cusp during the strain of labor. Luteal and rheumatic heart disease could be ruled out, and the diagnosis appeared quite certain. It was not possible to make a definite diagnosis of coarctation of the aorta in Case 7, although this diagnosis appeared to be correct. In the absence of the other signs of coarctation of aorta, the differences in blood pressure and the signs of the arteries of the legs could be explained on the basis of arteriosclerotic changes in them, although this is uncommon in her age group and occurs more frequently in men. All of her measurements of the circulation were in the normal range.

TABLE II

*Data relating to the circulation of nine cases of coarctation of the aorta\**

Case number	Date	Height	Weight	Body surface area	Oxygen consumption	Basal metabolic rate	Arteriovenous oxygen difference	Cardiac output	Cardiac output	Heart rate	Cardiac output	Cardiac area†	Cardiac volume‡	Cardiac work	Circulation** time (Docholín)	Circulation time CO <sub>2</sub>	Venous** pressure	Vital capacity	Red blood cells	Hemoglobin	Cardio-thoracic ratio‡
		cm.	kgm.	sq. m.	cc. per minute	per cent	cc.	liter per minute	liters per sq. m. per minute	per minute	cc. per beat	sq. cm.	cc.	gm. m. per beat	seconds	seconds	mm. saline	cc.	million per cu mm.	per cent	per cent
Average normal values§§							61.5		2.09		59				14.4		101				45.2
1	June 15, 1938 September 15, 1939	175.0 181.0	51.0 59.9	1.62 1.78	241 272	-6 -3	43.3 44.6	5.59 6.09	3.45 3.42	108 80	52 76	100.2 122.3	485.0 654.6	84.9 103.4	10.6 9.9	5.0 6.2	117 118	3500 4000	4.3	90	42 41
2	February 6, 1940	180.0	66.8	1.84	307	+5	49.6	6.19	3.36	68	91	132.3	733.5	163.4	13.5	4.8	50	5300	5.0	100	44
3	January 17, 1940	167.3	60.4	1.67	201	-4	61.7	3.26	1.95	60	54	110.2	531.8	80.8	14.1	6.7	72	2800	4.7	100	49
4	March 30, 1935	179.0	66.5	1.82	315	+24	90.3	3.52	1.93	89	41	284.2	2108.9	60.7	16.4		88	3250			59
5	September 23, 1939	165.0	48.3	1.52	257	+25	59.3	4.33	2.85	96	45	127.8	698.0	82.6	14.5		99	4100	5.5	116	45
6	September 30, 1939	156.7	52.2	1.50	218	+17	47.1	4.63	3.09	96	48	87.0	392.2	105.4	10.7	6.5	67	2700	5.1	102	44
7	January 30, 1940	162.5	54.8	1.51	172	-8	54.3	3.17	2.10	70	45	106.4	530.0	85.1	11.2	9.3	66	2300	4.6	100	49
8	December 29, 1939	183.0	74.6	1.97	224	-7	65.2	3.44	1.95	80	43	143.5	829.5	76.6	16.8	10.8	43	5100	4.0	94	46
9	March 10, 1940	181.5	69.3	1.91	216	-20	70.0	3.07	1.61	68	45	128.0	700.0	71.6	18.6	6.5	88	4400	5.5	115	46

\* See blood pressure records which go with these observations in Table I.

† The areas of the heart in Cases 10, 12, 13 and 14 were 83.5, 173.2, 174.2, and 128.1 sq. cm., respectively.

‡ The volumes of the hearts in Cases 10, 12, 13 and 14 were 370.0, 1101.3, 1111.9 and 701.2 cc., respectively.

\*\* The circulation time and venous pressure were 9.7 seconds and 104 mm., respectively, in Case 10.

† The cardiothoracic ratios in Cases 10, 12, 13 and 14 were 54, 59, 60, and 55, respectively.

§§ Stewart and Watson ((16) Tables II and III).

## OBSERVATIONS

The cardiac output was measured in nine (Cases 1 to 9) of the fourteen patients. In one of these (Case 1) observations were made on two occasions. None of these patients exhibited signs or symptoms of congestive heart failure. The data are recorded in Table II.

The cardiac output per square meter of body surface per minute (cardiac index) was increased in four cases (Cases 1, 2, 5, and 6) in whom the range was from 2.85 to 3.45 liters; it was within normal limits in four cases (Cases 3, 4, 7, and 8) with a range from 1.93 to 2.10 liters, and was reduced in one patient (Case 9) to 1.61 liters. The range of basal heart rate was from 68 to 108 beats per minute. The cardiac output per beat ranged from 41 cc. to 54 cc. in all but two cases. Case 2 had an output of 91 cc. per beat and Case 1, on the second estimation, fifteen months after the earlier one, had an output of 76 cc. per beat. During this interval there had been a gain in weight and in height, giving rise to an increase in surface area from 1.62 to 1.78 sq. m. The oxygen

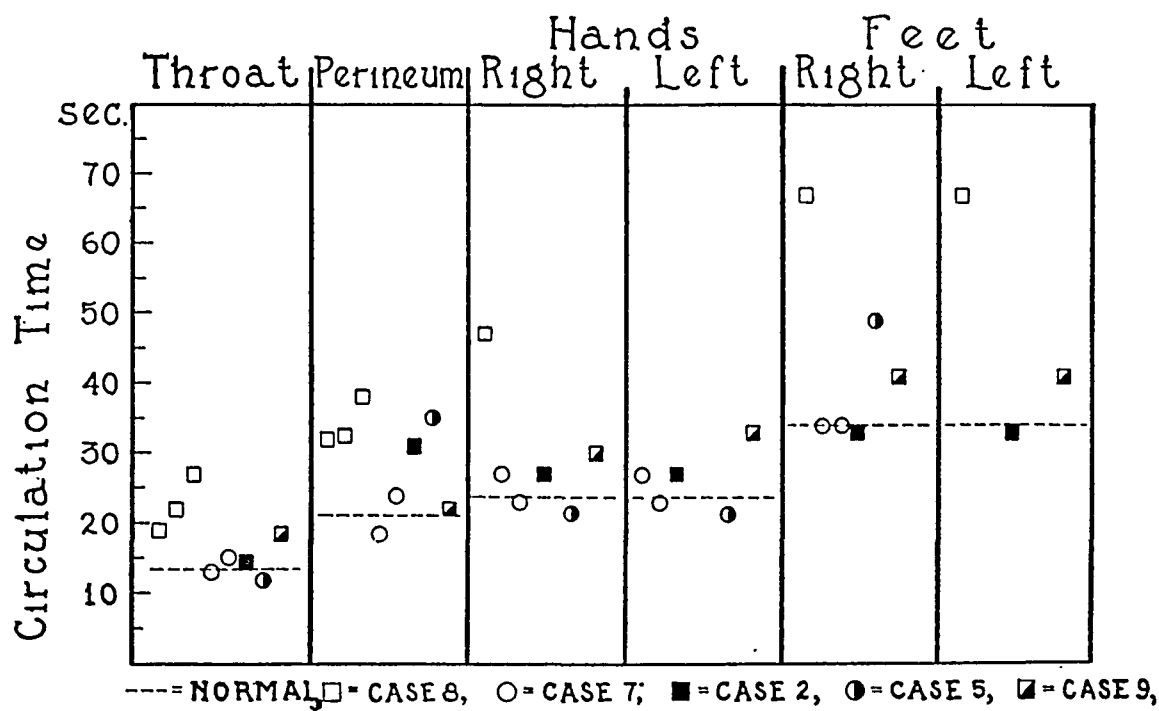
consumption increased slightly at the time of the second estimation but the basal metabolic rate was unchanged (6 per cent and 3 per cent, respectively). Both the arteriovenous oxygen difference and the cardiac index were unchanged.

In three of the nine patients the basal metabolic rate was increased to +24 per cent (Case 4), +25 per cent (Case 5), and +17 per cent (Case 6); in one it was decreased to -20 per cent (Case 9); and in the remaining five patients (Cases 1, 2, 3, 7, and 8) it was within the normal range.

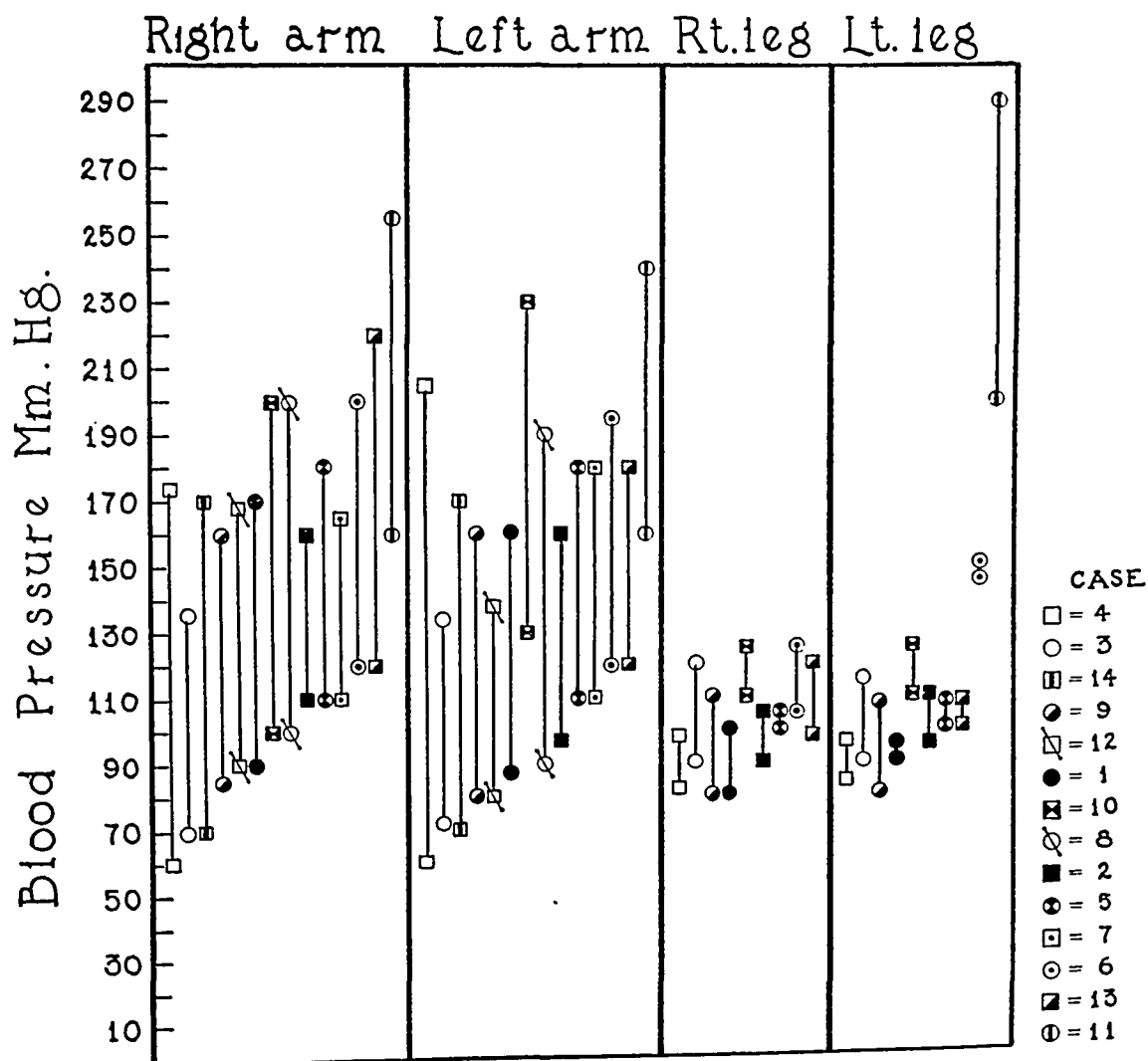
In four (Cases 3, 5, 7, and 8) of the nine cases the arteriovenous oxygen difference was within the normal range ( $60.0 \pm 5.0$ ),<sup>7</sup> in three (Cases 1, 2, and 6) it was decreased, and in two (Cases 4 and 9) it was increased.

According to x-ray photographs, the heart was increased in size with a cardiothoracic ratio of over 50 per cent in only one of the nine patients having measurements of cardiac output, and in

<sup>7</sup> Stewart and Watson (16) studied normal individuals under the same conditions and by the same methods.



**A**



**B**

FIG. 1

four of the other five patients (Table II). The left ventricular work ranged between 71.6 and 163.4 in grammeters per beat. When plotted against the heart size, the left ventricular work per beat was found to be adequate in all except Case 4 whose heart was large and in whom the cardiothoracic ratio was 59 per cent.

The arm-to-tongue circulation time (Decholin) was within normal limits (10.4 to 17 seconds (16)) in seven of the nine patients. In Case 10 it was slightly shorter than normal, measuring 9.7 seconds; and in Case 9 it was slightly prolonged, measuring 18.6 seconds. One patient (Case 8) had a prolonged circulation time throughout the parts of the vascular tree which were tested by Mascosol: throat, hands, perineum and feet (Figure 1A). In the other four cases (Cases 2, 5, 7, and 9) the arm-to-throat time was within or near normal limits. The arm-to-hand time was moderately prolonged in Case 9; the arm-to-perineum time was definitely prolonged in Cases 2 and 5; and the arm-to-foot time was prolonged in Cases 5 and 9.

The velocity of the blood flow from the lungs to the respiratory center was within or near normal limits in seven cases (Table II).

The venous pressure was slightly elevated in Case 1 and normal in the others (Table II).

#### DISCUSSION

The cardiac index was within the normal range or increased in all except one patient in whom it was decreased. The increased cardiac index in the two younger patients (Case 1 and Case 2) aged 15 and 17 years, respectively, is probably normal for their age (16). In them the basal metabolic rates were normal, and arteriovenous oxygen differences were small. In Case 5, having an increased cardiac index, the oxygen consumption was increased, and in Case 6 not only was the oxygen consumption increased, but the arteriovenous oxygen difference was small. In Case 9, having a decreased cardiac index, the basal metabolic rate was low and the arteriovenous

oxygen difference was wide. In this case also the arm-to-tongue circulation time was slightly longer than in the other patients (Figure 1A). In Cases 3, 7, and 8, having normal cardiac indices, both basal metabolic rates and arteriovenous oxygen differences were normal. In a fourth patient, Case 4, having a normal cardiac index, the basal metabolic rate and arteriovenous oxygen difference were both increased. This patient also exhibited increased basal metabolic rate when he was studied by Grollman and Ferrigan (2), but the arteriovenous oxygen difference was then normal. He died a few months after our observations were made (18); the increased arteriovenous oxygen difference no doubt indicated decrease in functional capacity of the heart (4, 19). The basal metabolic rate was increased in three of the nine patients. Grollman and Ferrigan (2) suggested that this phenomenon was occasioned by the increased blood supply to the thyroid gland. In none of the patients having increased oxygen consumption, however, was the circulation time shortened as is the case in hyperthyroidism.

In all except one (Case 4) of the nine cases, the heart at rest maintained an adequate output per beat in relation to its size. The cardiac output, as was discussed above, was within normal limits in this patient but the heart was greatly enlarged, and as a consequence the output was not in proportion to the cardiac size.

The velocity of blood flow was measured by three methods. To points above the level of coarctation it was within or near normal limits (Table II). The values of the arm-to-perineum and arm-to-foot circulation time with the solution described by Spier, Wright and Saylor (14) were prolonged in most instances but normal in a few cases (Figure 1A). Prolongation of the circulation time to the feet in a patient with normal circulation time to the throat and upper extremities is indicative of the devious route which blood must take in reaching the lower extremities. It may be recalled that Blumgart, Lawrence, and Ernestene (1), and, more recently, Woodbury, Mur-

FIG. 1. CIRCULATION TIMES AND BLOOD PRESSURES IN COARCTATION OF THE AORTA

In Figure 1A are recorded circulation times secured by using "Mascosol" in five patients suffering from coarctation of the aorta. The dotted lines show the average values for normal individuals. In Figure 1B are recorded the blood pressures in the four extremities of fourteen patients suffering from coarctation of the aorta.

phrey and Hamilton (20) found delay in the pulse wave in the lower extremities, as well as a prolonged circulation time to the feet.

Disproportion of blood pressure in the arms and legs was the most striking and constant finding in this group of patients (Table I, Figure 1B). It is recalled that in normal subjects both the systolic and diastolic levels of blood pressure are higher in the legs than in the arms. All patients except Case 11 showed a higher systolic pressure in the arms than in the legs. The diagnosis was not made during life in this patient (Case 11) and at autopsy only slight narrowing was found at the site of coarctation. Hydronephrosis, secondary to occlusion of the right ureter, and arteriolar nephrosclerosis were found at autopsy and may explain the elevated arterial pressure in the absence of more marked constriction of the aorta.

In our cases the relationship of the diastolic pressures in the arms and in the legs was variable. Blood pressure levels could not be obtained in the legs in four patients. Of the remaining ten patients, three exhibited higher diastolic pressure in the legs, four had approximately the same diastolic pressures in the arms and legs, and three showed higher diastolic pressure in the arms.

King's (21) analysis of the blood pressure readings in the reported cases of coarctation revealed that the blood pressure of the arms may be within the normal range. This was the situation in Case 3 in our series; nevertheless the systolic pressure in the legs bore the usual relationship found in coarctation; namely, it was lower. King found the systolic pressure in one or both arms uniformly higher than that in the legs. He found diastolic pressures of a higher level in the legs than in the arms six times. In our cases the systolic blood pressure readings were higher in the arms than in the legs in all except Case 11. The diastolic pressure in the legs was higher than that in the arms in three cases and was above 90 mm. in five cases. The levels of systolic and diastolic pressure in the arms and legs indicate that there is not only an increased resistance to the outflow of blood at the site of coarctation and in the collateral channels, but that there is a general increase in peripheral resistance sufficient to maintain a high diastolic pressure distal to the site of coarctation.

Steele (22) reviewed the knowledge of levels of arterial pressure in 203 cases of coarctation of the aorta and, by intra-arterial measurement, found the diastolic pressure to be elevated above 100 mm. Hg in the femoral as well as the radial arteries in two of three cases he studied. He concluded that the increase in peripheral resistance need not be confined to the upper half of the body alone, that there may often be general increase in arteriolar tone throughout the body, and that, under these circumstances in so far as the distribution of peripheral arteriolar resistance is concerned, the arterial hypertension in coarctation of the aorta does not differ from the common forms of arterial hypertension. Our cases herein reported, in which the blood pressure in all extremities was increased, point to the same conclusion.

The absence of palpable pulsations in the arteries of the legs was an inconstant finding. Eight cases had palpable pulsations over the femoral arteries, and in four of these pulsations were also palpable over the popliteal and dorsalis pedis arteries. The pulsations were feeble, however. Pulsating superficial and intercostal arteries were palpable over the upper thorax posteriorly in eleven of the fourteen patients. In six of these, bruits were heard over the dilated arteries (Table I).

X-ray examination of the chest revealed erosion of the ribs with scalloping and nicking of the inferior margins posteriorly in eleven of our fourteen cases, which was the most characteristic x-ray finding (Table I). Five cases showed defects in the aortic arch. This abnormality was not searched for in oblique films in all cases. One patient had absence of the aortic knob and one had a prominent aortic knob; both showed scalloping of the ribs.

Although these patients had hypertension and had performed an increased amount of work per beat over long periods, the heart, as ascertained from the cardiothoracic ratio, was enlarged in only one of the nine cases (Case 4) in whom the cardiac output was measured, and in four (Cases 10, 12, 13, and 14) of the other cases studied; in the remaining one (Case 11) the film was misplaced before this measurement had been made. Four of the five cases having large hearts died during the seven-year period which these obser-

ratios covered. Enlargement of the heart, then, appears to indicate a poor prognosis.

Electrocardiograms did not show any pathologic changes. In eleven of the fourteen cases there was deviation of the electrical axis to the left, a finding which causes no surprise because of burden placed on the left heart by the narrowing of the atria and because of hypertension (Table I). In seven patients, the T-waves of the electrocardiogram were abnormal and appeared to indicate myocardial damage. Since these studies were limited, five of these seven have died. The occurrence of T-wave changes indicates a poor prognosis.

#### SUMMARY AND CONCLUSIONS

1. This study concerns fourteen cases of obstruction of the atria. The diagnosis was made during life in thirteen of these, at autopsy in one case, and in four cases who died some time after the diagnosis had been made it was confirmed by autopsy.

The following observations were made in nine of these cases: cardiac output, basal metabolic rate, arteriovenous oxygen difference, cardiac output per beat, left ventricular work per beat, the blood pressure in all four extremities, venous pressure, vital capacity, circulation time by means of dextrin, carbon dioxide inhalation, and Maschke's, electrocardiograms and x-rays of the chest (Tables I and II). Clinical observations of five other patients are included in Table I.

2. The heart at rest was found to maintain a normal or even increased volume output of blood in obstruction of the atria before the onset of failure. In only one of the nine cases was the cardiac output decreased (Case 9). In only one of these patients (Case 4) was the heart enlarged to give a cardiothoracic ratio greater than 50 per cent. The work of the heart in relation to the cardiac size was, as a consequence, adequate in all except this one case (Case 4). This patient had a cardiac output within normal limits but it was not in proportion to the greatly enlarged heart.

3. The systolic blood pressure in the arms was higher than that in the legs in all except one patient. This finding was the most constant of the characteristic signs of obstruction of the atria. In most cases, there was not only an increase in

the resistance to the outflow of blood at the site of obstruction and in the collateral channels, but also a generalized increase in peripheral resistance sufficient to maintain a high diastolic pressure below the level of the obstruction.

4. The circulation time was within or near normal limits above the level of obstruction but there was a tendency to prolongation below the obstruction.

5. Enlargement of the inferior margins of the ribs posteriorly, giving rise to scalloping and notching, was the most common x-ray finding in obstruction of the atria. This was noted in eleven of the fourteen cases.

6. Enlargement of the heart and T-wave changes in the electrocardiogram point to poor prognosis when they occur.

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# LYSOLECITHIN AND HEMOLYTIC ANEMIA. THE SIGNIFICANCE OF LYSOLECITHIN PRODUCTION IN THE DIFFERENTIATION OF CIRCULATING AND STAGNANT BLOOD<sup>1</sup>

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Considerations regarding the etiology of hemolytic jaundice are inextricably bound up with the presence of spherocytosis and the associated increase in hypotonic saline fragility, and the effect of splenectomy in the amelioration of this disorder. Although many different opinions are advanced regarding the etiology of the disease, the outstanding ones are those postulating a faulty bone marrow or an increase in hemolysin production. The theory of faulty bone marrow production, as introduced by Naegeli (1), claims that spherocytes are produced by the bone marrow and that splenectomy does not change the production of abnormal red cells. The dramatic effect of this operation is explained by removal of the inhibitory effect of the spleen on the bone marrow (1). Careful studies of the hemoglobin metabolism in this disorder, however, have revealed that, after splenectomy, increased blood destruction (as measured by the urobilinogen output) and also increased erythrocyte production (as evaluated by the reticulocyte counts) return to normal values despite the continued presence of spherocytes. Some observers (2) believe that the spleen is the most important organ in this disease but do not deny production of spherocytes by the bone marrow.

The hemolysin theory, on the other hand, states that the shape of the red cells is altered outside the bone marrow through the action of hemolysins. Dameshek and Schwartz (3) produced spherocytosis in experimental animals by the injection of specific hemolytic sera and Haden (4) was able to induce the same abnormality *in vitro* by the addition of hypotonic saline solutions to normal erythrocytes. The former authors have

interpreted spherocytosis as a precursor of hemolysis and the morphological expression of an alteration in the structure of erythrocytes due to the action of different types of hemolytic substances. Dameshek and Schwartz (3) demonstrated the presence of hemolysins of the immune body type in cases of acquired hemolytic anemia and showed the disappearance of these antibodies and of spherocytosis after splenectomy. In congenital hemolytic anemia, however, the demonstration of hemolysins has not been consistently made. Recently, Bergenhem and Fåhræus (5) showed the presence in normal blood serum of a hitherto unknown hemolytic substance called lysolecithin. They postulated that an increase in the production of this lysin might be the cause of congenital hemolytic anemia. Singer (6) demonstrated the increased fragility of spherocytes present in the congenital condition towards this lysin but found that spherocytes from cases of acquired hemolytic anemia reacted like normal cells. These findings have recently been confirmed by Gripwall (2). The present paper deals with the problem of lysolecithin production in the body and the possible relationship of this physiological lysin to the development of congenital hemolytic jaundice.

## THE RESEARCHES OF BERGENHEM AND FÅHRAEUS

Bergenhem and Fåhræus (5) were led to the discovery of their lysolecithin by observing characteristic changes in blood which was allowed to stand quietly for several hours at body temperature. This incubated blood showed considerable retardation of the sedimentation rate of the erythrocytes, a change in the shape of red cells from the biconcave to the spherical form and a noticeable reduction of aggregation (rouleaux formation). These authors demonstrated that these changes were due to the action of a substance

<sup>1</sup> This work has been aided by grants from the Milton fund of Harvard University and the Dazian Foundation for Medical Research.



formed by an enzyme which was capable of splitting this substance from the serum lipoids. Adsorption of the latter substance by the red blood cells resulted in the above alterations. If stronger concentrations of this substance developed, actual hemolysis of the erythrocytes was noted. The enzymatic process in the serum reached its maximum at a temperature of 42° C. and a pH of 7.2 and was inactivated at 56° C. Bergenheim and Fåhræus (5) concluded that this hemolytic substance was either identical with the so-called lysolecithin or very closely related to it.

Lysolecithin is a definite chemical substance<sup>2</sup> which has previously received attention in the study of the biological effect of snake venom. From the investigations of Flexner and Noguchi (8), Kyes and Sachs (9), Luedecke (10), Manwaring (11), Delezenne and Ledebt (12), it is known that cobra toxin hemolyzes red blood cells only in the presence of serum and that the blood lipoids are necessary for this hemolytic activity. Snake venom also contains a lecithinase which changes lecithin to lysolecithin, the latter having hemolytic qualities. Small amounts of lysolecithin obtained by the action of cobra toxin on lecithin, when added to normal blood, produce the same changes as the substance obtained from incubated serum. If normal serum is inactivated, and cobra toxin then added, the hemolytic power of the serum extract can be reestablished. Formation of lysolecithin by the lecithinases present in cobra toxin and in normal serum can be prevented by adding quinine which is a typical inhibitor of the action of lipases.

Recent investigations by Bergenheim (7) have revealed certain other qualities of lysolecithin. This author pointed out that lysolecithin is in some manner related to the complement function of the serum and that there are several parallelisms in the behavior of this lysin and of complement which are of particular interest. It has been known for many years that such manipulations as *shaking* of the serum, dilution with distilled water, etc. may destroy or inactivate the complement function (*cf.* Sachs and Klopstock (13)). Ber-

genhem and Fåhræus (5) demonstrated that lysolecithin is also destroyed or rendered inactive by shaking or moving the serum. The most interesting phenomenon was the demonstration that the lysolecithin content present in a freshly drawn blood sample can be increased if the *unmoved* serum is incubated for several hours; if, however, the serum is kept in motion during incubation, this increase does not occur. Since circulating blood is under conditions of constant motion, it is to be expected that the amount of lysolecithin present in the circulating blood must be smaller than in blood which is stagnant.

Bergenheim and Fåhræus (5) believe that lysolecithin may be of great importance in the physiological mechanism of erythrocyte destruction. They point out that the spleen must be considered as an organ containing a more or less constant reservoir of blood, as shown by the well-known researches of Barcroft (14) and his associates. Blood stored in the spleen is temporarily excluded from the circulation, and in this splenic reservoir an increased formation of lysolecithin may occur. By adsorption of this substance by the erythrocytes, direct hemolysis of the red blood corpuscles in the spleen, or preparation of these erythrocytes for further destruction, presumably takes place. In hemolytic jaundice, where the enlarged spleen contains large quantities of blood, an increased production of lysolecithin is said to occur and is held to be responsible for the increased erythrocyte destruction. For lack of a biological micromethod for determination of lysolecithin, Bergenheim and Fåhræus (5) were unable to carry out direct measurements of this substance in blood. They therefore worked with large quantities of horse serum, measuring changes in the red cells by means of the sedimentation rate. As changes in the sedimentation rate are frequently slight and difficult to evaluate, a simpler and more accurate method for determination of lysolecithin appeared desirable. In another paper (6) a method for determination of the lysolecithin fragility of various red cells (normal and spherocytic) was described. By adaptation of this method the problem of the significance of lysolecithin in the mechanism of hemolysis in physiological and pathological conditions was facilitated.

<sup>2</sup> Lysokephalin is supposed to have quite similar qualities. Both lysolecithin and lysokephalin are sometimes called lysocithin (7).

## METHODS

In the paper (6) on the lysolecithin fragility test, a method for extraction of this lysin from serum was described. This method has been adapted for the quantitative determination of the lysolecithin content of serum. Blood is drawn and put into the ice chest until it is completely clotted and the serum is expressed. Then the serum is removed from the clot by means of a capillary pipette. Usually, the obtained serum is centrifuged in order to remove the last vestiges of erythrocytes. It was noted that the process of centrifuging the serum for several minutes apparently does not diminish the content of lysolecithin. The serum, which is now completely freed from any erythrocytes, is then divided into two equal parts. One part is precipitated immediately by means of alcohol, the other part after incubation. Ten cc. of blood serum are precipitated with 10 times its quantity of 95 per cent alcohol, filtered, and the filtrate evaporated in a vacuum apparatus at room temperature to complete dryness. The residue is again dissolved in 5 cc. alcohol, filtered, mixed with 6 times its quantity of ether, and placed in the refrigerator overnight. The precipitate contains the lysolecithin. After centrifugation the ether is removed by means of a suction pump, and after the precipitate is dried the content of lysolecithin is determined. For this determination its hemolytic power is used. The dried precipitate is now dissolved in 0.8 cc. of physiological salt solution. From this basic solution a geometric series of dilutions is prepared in the usual manner by further dilution with saline. An approximate 2 per cent saline suspension of washed erythrocytes<sup>3</sup> (containing about 100,000 cells per cmm.) is taken up in leukocyte pipettes to the 1 mark and diluted with the various single dilutions of lysolecithin to the 11 mark. While hemolysis is taking place, the pipettes are set aside for one hour; with very weak concentrations of the lysin present, the reaction may be facilitated by placing the pipettes in the incubator for the same period. The solutions are then examined in a hematocytometer. By determining the number of undissolved erythrocytes, an exact measure of the degree of hemolysis is obtained. Hemolysis above 90 per cent is regarded as complete. At this degree of hemolysis the opaque fluid in the pipettes becomes clear and transparent. The lysolecithin content of a serum is expressed in the hemolyzing value of the substance derived from 10 cc. of serum. Only the solutions which give complete hemolysis are considered for the evaluation.

As already mentioned, Bergenhem and Fåhræus (5) found that the lysolecithin content of a serum

<sup>3</sup> The erythrocyte suspension is usually made of the patient's own red cells, but it is also possible to use other normal erythrocytes as there is practically no difference in the lysolecithin fragility of the erythrocytes of normal persons. These erythrocytes are obtained from oxalated blood and are then washed three times with normal saline before suspension is prepared.

can be increased if unmoved blood is incubated for from 8 to 24 hours, but that this increase does not take place if blood is moved during the incubation period. As an example, Table I shows the results of determination in the lysolecithin content of 2 aliquot samples of unincubated and incubated serum in 2 different dogs and also the results of movement during the whole incubation period.

TABLE I

*Lysolecithin content of unincubated and incubated serum*

Dog number	Kind of serum	Lysolecithin content (expressed as hemolyzing value or titer)								Hemolyzing value of		Lysolecithin quotient
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	Unincubated serum	Incubated serum	
6	Unincubated hemolysis, per cent	+	+	+	-	-	-	-	-	1:8		1:8
	Incubated: * Unmoved hemolysis, per cent	+	+	+	+	+	+	-	0		1:64	
	Moved † hemolysis, per cent	-	-	-								
		52	0									
8	Unincubated hemolysis, per cent	+	+	+	+	+	-	-	-	1:32		1:4
	Incubated: * Unmoved hemolysis, per cent	+	+	+	+	+	+	+	-		1:128	
	Moved † hemolysis, per cent	-	-	-								
		58	0									

\* Time of incubation was 20 to 24 hours.

† Shaking of the serum was performed by means of a motor twice a minute.

These experiments confirm the statement of Bergenhem and Fåhræus (5). In determining the relationship between the lysolecithin content of 2 equal amounts of unincubated and incubated blood of the same blood sample, we should be able to differentiate between stagnant (*i.e.* unmoved) and circulating (*i.e.* moved) blood. In *stagnant* blood we can expect that the lysolecithin content of the unincubated blood sample must be much higher compared with the corresponding value in circulating blood, because this blood reacts like the blood sample in the incubator. The relationship of the lysolecithin content in equal amounts of unincubated and incubated unmoved blood of

the same sample is called the lysolecithin quotient (LLQ):

$$\frac{\text{Lysolecithin content of unincubated serum}}{\text{Lysolecithin content of incubated serum}} = \text{LLQ.}$$

In stagnant blood we should find a LLQ of 1 or approaching 1, in contradistinction to circulating blood where the LLQ must be smaller due to the fact that the amount of lysolecithin can be increased by incubation. In stagnant or stored blood this incubation has already taken place in the body. In the following experiments the LLQ was always determined from 10 cc. of serum. To use smaller quantities of serum, the method has been further modified: the test is made in special pipettes that take just half of the content of normal leukocyte pipettes. The amount of serum used for each determination can thus be reduced to 5 cc.; the dried lysolecithin for each evaluation is dissolved in 0.4 cc. instead of 0.8 cc. of normal salt solution.

## RESULTS

### A. The LLQ of peripheral blood in normal conditions

The LLQ was determined in 8 dogs and 30 normal human beings. The normal group comprised healthy physicians, students and patients who showed no evidence of liver, spleen, or blood disease or of any condition in which an abnormally lowered velocity of blood flow was suspected. Table II contains the values obtained in 10 human beings and the first column of Table III shows the results in dogs. For simplification, only the end results of the determinations are given. The lysolecithin content of dog serum was tested against dog erythrocytes, but for the determination of lysolecithin in human serum, human erythrocytes were used. The hemolytic titer of dog serum is apparently greater than that of human serum, although this may simply be due to the greater fragility of the red cells of the dog to lysolecithin. Normally the LLQ is always a fraction of 1, the lysolecithin content (expressed in hemolytic titer) of the incubated serum being 2 to 8 times greater than that of the unincubated serum. Relatively great differences were encountered in different individuals. In human

beings only venous blood was used for the determinations. In dogs no difference was demonstrated in the LLQ of arterial as against venous blood of the same animal, but rather considerable individual differences were present. The LLQ in the 8 dogs was always  $\frac{1}{4}$  to  $\frac{1}{8}$ .

TABLE II  
LLQ in normal human blood

Number	Lysolecithin content of serum		LLQ
	Unincubated	Incubated	
1	1:2	1: 8	2: 8 = $\frac{1}{4}$
2	1:4	1: 8	4: 8 = $\frac{1}{2}$
3	1:4	1:16	4:16 = $\frac{1}{4}$
4	1:8	1:16	8:16 = $\frac{1}{2}$
5	1:2	1:16	2:16 = $\frac{1}{8}$
6	1:4	1:16	4:16 = $\frac{1}{4}$
7	1:4	1:32	4:32 = $\frac{1}{8}$
8	1:4	1: 8	4: 8 = $\frac{1}{2}$
9	1:8	1:16	8:16 = $\frac{1}{2}$
10	1:2	1:16	2:16 = $\frac{1}{8}$

### B. The LLQ in splenic vein blood (stored blood)

Determinations of the LLQs in blood obtained from the splenic artery and vein were performed in 8 dogs in order to show possible differences between the "reservoir blood" of the spleen and that of the circulating peripheral blood. Due to the great difficulties in obtaining sufficient amounts of reservoir blood directly from the spleen, determinations in the splenic vein blood were made because it was thought that the splenic vein might not only contain blood passing directly through this organ, but also a more or less large quantity of stored blood. The results are tabulated in column 2 of Table III.

In all experiments the LLQ in the splenic vein blood was greater than that in the splenic artery (*i.e.*, following incubation of splenic vein blood there was usually no increase in lysolecithin titer). In 6 of the 8 experimental animals the unincubated blood of the splenic vein contained as much lysolecithin as the incubated; the LLQ was 1. In 2 animals the lysolecithin content of the unincubated sample was not an optimal one and could still be increased by incubation. But compared with the corresponding arterial blood, a higher lysolecithin content was observed in these 2 dogs as well. In order to decide whether the erythrocytes coming from the stored blood of the

TABLE III  
LLQ in dog serum

Dog number	Lysolecithin content in serum from								
	Peripheral blood*			Splenic vein blood			Hepatic vein blood†		
	Un-incubated	Incubated	LLQ	Un-incubated	Incubated	LLQ	Un-incubated	Incubated	LLQ
1	1: 8	1: 64	$\frac{1}{8}$	1:64	1: 64	1	1: 8	1: 64	$\frac{1}{8}$
2	1: 8	1: 32	$\frac{1}{4}$	1:32	1: 32	1	not determined		
3	1:16	1: 64	$\frac{1}{4}$	1:64	1: 64	1	not determined		
4	1:16	1: 64	$\frac{1}{4}$	1:64	1: 64	1	not determined		
5	1: 8	1: 64	$\frac{1}{8}$	1:64	1: 64	1	not determined		
6	1: 8	1: 64	$\frac{1}{8}$	1:64	1: 64	1	1: 8	1: 64	$\frac{1}{8}$
7	1:32	1:128	$\frac{1}{4}$	1:64	1:128	$\frac{1}{2}$	not determined		
8	1:32	1:128	$\frac{1}{4}$	1:64	1:128	$\frac{1}{2}$	1:32	1:128	$\frac{1}{4}$

\* Peripheral blood was drawn from jugular vein or carotid artery.

† Hepatic vein blood was obtained after preceding ligation of inferior vena cava.

spleen might have already adsorbed lysolecithin with a resultant tendency to spherocytosis and increased fragility, the lysolecithin fragility of red cells from the splenic vein and the splenic artery was compared. Bergenhem and Fähræus (5) noted such a tendency, and Heilmeyer (15) found by direct measurement of the thickness of the red cells from the splenic artery and the splenic vein in patients with hemolytic anemia that the splenic vein erythrocytes were considerably thicker. Such a tendency should lead to increased fragility. Table IV (as an example of numerous experiments) shows, however, that no difference in the lysolecithin fragility of the erythrocytes in both splenic vessels could be demonstrated. In order to test the lysolecithin fragility, 2 exactly equal suspensions of erythrocytes from the splenic artery and the splenic vein (100,000 cells per cmm.) were tested against 2 exactly equal concentrations of a lysolecithin extract.

TABLE IV  
Lysolecithin fragility of splenic artery and splenic vein erythrocytes

Kind of erythrocytes	Lysolecithin dilutions					
	1:2	1:4	1:8	1:16	1:32	1:64
From artery: hemolysis, per cent	+	+	+	+	—	0
From vein: hemolysis, per cent	+	+	+	+	—	0
	100	100	100	94	38	0
	+	+	+	+	—	0
	100	100	100	92	35	0

### C. The LLQ in hepatic vein blood

To decide whether blood coming from other blood reservoirs than the spleen might show the same changes, the LLQ in hepatic vein blood obtained after ligation of the inferior vena cava was determined in 3 dogs (Table II, column 3). Some investigators (16, 17) believe that not only the spleen but also the liver may be regarded as an organ containing more or less large blood depots. In contradistinction to the splenic vein blood, hepatic venous blood showed the same LLQ as found in the peripheral circulation.

### D. The LLQ in the blood of varicose veins

As already pointed out, a LLQ of 1 may, under certain conditions, demonstrate the presence of stagnant blood, or at least of a very sluggish circulation. To confirm this interpretation, the LLQ was determined in other conditions in which the presence of a sluggish circulation of blood might be assumed. Following a suggestion by Dr.

TABLE V  
The LLQ in varicose vein blood

Case number	Lysolecithin content in serum from					
	Cubital vein blood			Varicose vein blood		
	Unincubated	Incubated	LLQ	Unincubated	Incubated	LLQ
1	1:8	1:16	$\frac{1}{2}$	1:16	1:16	1
2	1:8	1:16	$\frac{1}{2}$	1:16	1:16	1
3	1:8	1:16	$\frac{1}{2}$	1:16	1:16	1
4	1:8	1:16	$\frac{1}{2}$	1:16	1:16	1
5	1:4	1:16	$\frac{1}{4}$	1:16	1:16	1
6	1:2	1: 8	$\frac{1}{4}$	1: 8	1: 8	1
7	1:4	1: 8	$\frac{1}{2}$	1: 4	1: 8	$\frac{1}{2}$

Robert Brandt of Augusta, Georgia, determinations of the LLQ in blood from varicose veins were performed. The investigations of Magnus (18) have shown that the blood in large varicose veins possesses a very sluggish circulation, particularly when the patient is in a horizontal position. Nobel (19) and Wolf and Remenovskiy (20) demonstrated this tendency to stagnation by x-ray studies of the injected veins. In 6 patients with very large varicose veins the LLQ in blood from the cubital vein and in blood from the varicose vein was compared. Blood was taken in the morning before the patient got up. Table V

shows that blood obtained from the varicose veins always presented a LLQ of 1 in contradistinction to blood from the cubital veins. Case 7 shows identical values for blood of the upper and lower extremities; no varices were present and this case was taken as a control.

### *E. The LLQ in cases of congenital hemolytic anemia*

According to the hypothesis of Bergenhem and Fåhræus (5), lysolecithin may be considered to be the lysin responsible for the spherocytosis and increased blood destruction in this disorder. It seemed, therefore, of great interest to determine the LLQ in such cases. Table VI shows the results obtained in 6 cases of congenital hemolytic jaundice.

TABLE VI  
*The LLQ in cases of hemolytic jaundice*

Case number	State of blood	Lysolecithin content of serum* from		
		Unincubated blood	Incubated blood	LLQ
1	Hemoglobin 50% Erythrocytes 2,500,000 Reticulocytes 9%	1:1	1:16	$\frac{1}{16}$
2	Hemoglobin 54% Erythrocytes 3,000,000 Reticulocytes 7.8%	1:8	1:16	$\frac{1}{2}$
3	Hemoglobin 90% Erythrocytes 4,200,000 Reticulocytes 8.4%	1:2	1: 4	$\frac{1}{2}$
4	Hemoglobin 64% Erythrocytes 3,600,000 Reticulocytes 8.2%	1:4	1: 8	$\frac{1}{2}$
5	Hemoglobin 70% Erythrocytes 3,600,000 Reticulocytes 18%	1:4	1:16	$\frac{1}{4}$
6	Hemoglobin 106% Erythrocytes 4,900,000 Reticulocytes 7.2%	1:8	1:16	$\frac{1}{2}$

\* Blood was obtained from the vein of the antecubital fossa.

In all cases the saline fragility of the erythrocytes was increased and the lysolecithin fragility test was likewise pathological. The evaluation of the lysolecithin content was performed with normal erythrocytes in order to avoid errors which might be caused by the increased susceptibility of

the patient's own erythrocytes. As demonstrated in another paper (6a), the spherocytes of congenital hemolytic jaundice have a decreased resistance towards lysolecithin and therefore give higher hemolyzing values. All these cases show no great difference from the normal cases as far as the lysolecithin content of the serum is concerned. Only Case 1 had a very low value in the unincubated blood. In no case was there any evidence indicating an increased production of lysolecithin.

### DISCUSSION

The results thus far obtained have confirmed the findings of Bergenhem and Fåhræus (5) regarding lysolecithin. The demonstration has furthermore been made that the concentration of this physiological lysin is increased by keeping the serum quietly in the incubator for several hours; no such increase occurs in serum which has been shaken. This increase in the amount of lysolecithin in incubated serum may be expressed by the lysolecithin quotient, in which the relationship of the lysolecithin content of unincubated to that of incubated (unmoved) serum of the same blood sample is determined.

In circulating blood this quotient is always a fraction of 1. In stagnant blood the LLQ is 1 or approximates 1, probably because the enzymatic process causing an increase in lysolecithin in the incubated test tube has already been active within the body. In splenic vein blood of dogs the LLQ was found to be 1 in most animals, probably due to storage of blood in the spleen. At least from the standpoint of lysolecithin production, the spleen may be considered to be the "incubator" of the body. That a LLQ of 1 is the serological manifestation of stagnant blood was also indicated in investigations of blood obtained from varicose veins. The extremely sluggish circulation in these veins, particularly in patients placed in a horizontal position for several hours, has been demonstrated by Magnus (18), Nobel (19) and others (20). The finding of a LLQ of 1 in blood from these veins in contradistinction to a LLQ of  $\frac{1}{16}$ ,  $\frac{1}{4}$ , etc. in blood obtained from the cubital vein in the same patient supports the interpretation suggested in this paper.

The concept of Rein (16) and associates, Ep-

pinger (17), and others, that the liver may also be regarded as an organ containing blood depots, could not be corroborated by determining the LLQ. The finding of a normal LLQ in the hepatic veins may be explained either by the assumption that no "storage" blood was obtained in our experiments, or that the mechanism of storage of the blood in the liver may be quite different from that in the spleen. The recent investigations of Knisely (21) on the histology of the spleen indicate that the red cells are separated from the fluid of the blood in the venous sinuses of the spleen. As a LLQ of 1 was obtained from serum of the splenic vein blood in 6 of 8 examined animals, we may conclude that not only the erythrocytes but also the plasma must be stored in the spleen for the reason that only unmoved serum gives a LLQ of 1. Due to this storage the "reservoir blood" of the spleen probably has an increased content of lysolecithin.

Although these experiments confirm the hypothesis of Bergenhem and Fåhræus (5) that splenic reservoir blood contains more lysolecithin than circulating blood, there is at present no proof for the assumption that, since this substance has hemolytic power when extracted from the serum, it is therefore actively engaged in normal blood destruction. It is also very doubtful whether the concentration of lysolecithin obtainable from serum may be considered sufficient for the range of normal blood destruction in the body. The recently published experiments of Ham and Castle (22), who believe that splenic hemolysis is due to metabolic changes of the erythrocytes produced by intravascular stasis, likewise rule out any action of lysolecithin in this mechanism. Furthermore, lysolecithin can by no means be regarded as the lysis primarily responsible for the hematological changes in congenital hemolytic jaundice. According to the hemolysin theory, spherocytosis is produced outside of the bone marrow by action of hemolysins upon normal red cells. It is the morphological expression of an alteration in the physical-chemical structure of erythrocytes. Although in a previous communication (6a) demonstration was made that the lysolecithin fragility of spherocytes in congenital hemolytic jaundice is remarkably increased compared with that of normal cells, the *lysolecithin production* in the pe-

ripheral blood in that disorder was found to be normal.<sup>4</sup> In order to explain the presence of spherocytosis and greatly increased blood destruction regularly seen in congenital hemolytic jaundice, Bergenhem and Fåhræus (5) assumed an increased production of lysolecithin. The findings of both normal content and quotient appear to rule out the lysolecithin as of any significance in the pathogenesis of this disease. We believe, therefore, that lysolecithin, although it has hemolytic qualities, is not the hemolysin responsible for the production of hemolytic jaundice.

#### SUMMARY

1. Bergenhem and Fåhræus demonstrated that normal blood contains a hemolyzing substance called lysolecithin. They believed that this lysis was developed in high concentration in the stored blood of the spleen and was therefore involved in the physiological mechanism of blood destruction. Congenital hemolytic jaundice was assumed to be due to an increased production of this substance.

2. A quantitative method is described for determination of the lysolecithin content in relatively small amounts of blood. The content of lysolecithin in peripheral blood of dogs and normal human beings was determined. It was demonstrated that this content could be increased 2 to 8 times by incubating unmoved blood for several hours, but that moving the blood during incubation did not produce this increase, probably due to the fact that moving destroyed or inactivated the lysis. Circulating (*i.e.* moved) blood contains less lysolecithin than stagnant (*i.e.* unmoved) blood. To relate circulation of blood to its lysolecithin production the lysolecithin quotient  $LLQ =$

$$\frac{\text{Lysolecithin content of unincubated serum}}{\text{Lysolecithin content of incubated serum}}$$

was determined.

3. The LLQ of normal peripheral blood is always a fraction of 1; in stagnant or stored blood the LLQ was found to be 1. By means of the LLQ, the differentiation between circulating and stagnant blood appears possible.

<sup>4</sup>Unfortunately, no data concerning the lysolecithin production in splenic artery and splenic vein blood in cases of hemolytic jaundice are yet available, although such investigations would be highly desirable.

4. Blood from the splenic vein in dogs has a LLQ of 1, indicating the presence of stored blood coming from the spleen. The LLQ in blood from the splenic artery, however, was always a fraction of 1. These findings demonstrate an increased lysolecithin production in the "splenic reservoir blood."

5. The LLQ obtained from blood from varicose veins, presenting an area of very sluggish circulation, was likewise 1.

6. The LLQ in blood from the hepatic vein was the same as in peripheral blood. This finding might indicate that the mechanism of "storage of blood" in the liver might be different from that in the spleen, where, presumably, separation of plasma and erythrocytes inside the organ occurs (21).

7. No difference in the lysolecithin content and the LLQ in the blood of patients with congenital hemolytic anemia, as compared with that of normal persons, was found.

8. The significance of lysolecithin as the physiological hemolysin involved in the mechanism of normal blood destruction by increased production in splenic reservoir blood is not conclusive. It is also very unlikely that lysolecithin can be regarded as responsible for the increased blood destruction in congenital hemolytic jaundice, since no increase in the production of this lysin was demonstrable in the disease. It is therefore believed that lysolecithin, although having hemolytic qualities, is not the hemolysin causing hemolytic anemia.

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# THE PATHOGENESIS OF AZOTEMIA IN HEMORRHAGE FROM THE UPPER GASTRO-INTESTINAL TRACT<sup>1</sup>

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In 1934 Sanguinetti (1) observed a rise in the blood urea after hemorrhage into the gastro-intestinal tract. This observation has been confirmed in many subsequent reports. It has been shown that not all patients with comparable degrees of hemorrhage from the upper gastro-intestinal tract develop azotemia. The reason for this disparity has not been clarified. Several theories, none of which has been universally accepted, have been proposed to explain the pathogenesis of post-hemorrhagic azotemia: (1) Toxic destruction of body protein (1, 2, 3, 12); (2) toxicosis due to bacterial decomposition of the stagnant blood (1, 4); (3) absorption of digested blood (1, 5, 6, 7); (4) abnormality of chloride metabolism (6, 8); and (5) functional impairment of renal activity (6, 7, 9, 10, 12, 13, 14).

This communication reports a study of 17 patients with upper gastro-intestinal hemorrhage. Our observations support the opinion that azotemia following hemorrhage into the stomach and upper part of the small intestines occurs only when the kidney function is temporarily or permanently reduced. Evidence is presented which indicates that such azotemia is due to an accentuation and prolongation of the normal physiological rise in the blood nonprotein nitrogen which follows the ingestion of a large amount of protein.

## GENERAL PROCEDURE AND METHODS

Blood for chemical analysis, hemoglobin, and erythrocyte counts was taken on admission to the hospital and at frequent intervals as indicated in the tables. Renal function was studied by repeated measurement of the urea and creatinine clearances. In most instances, the initial observations on renal function were begun within a few hours after admission to the hospital. The blood urea nitrogen was measured by the manometric urease

method of Van Slyke (19). Blood serum chloride (blood collected under oil) was determined by the method of Van Slyke and Sendroy (19). Nonprotein nitrogen was determined by the micro Kjeldahl and direct nesslerization method of Wong (19). Plasma carbon dioxide combining power was measured by the method of Van Slyke and Cullen (19). Creatinine clearances were done according to the method of Holten and Rehberg (22). Urea clearances were done by the method of Möller, McIntosh, and Van Slyke (21). The urea clearance was calculated by the method of Chesley (16) when the rate of urine excretion was less than 0.35 cc. per minute. In practically all cases the urine collection was made by catheter. Values for blood and urine urea are expressed as urea nitrogen.

## SUMMARY OF CLINICAL DATA

Table I is a summary of the 17 cases. The maximum blood urea nitrogen and nonprotein nitrogen, day of admission after onset of hemorrhage, the severity of hemorrhage as estimated by the red blood cell count, blood pressure, and renal

TABLE I  
Summary of 17 cases of upper gastro-intestinal hemorrhage

Case number	Admission day*	Age Sex	Blood urea nitrogen mgm. per cent	Nonprotein nitrogen mgm. per cent	Blood chloride m. eq.	Plasma CO <sub>2</sub> C.P. vol-umes per cent	Clearance		Admission			Lowest blood pressure
							Urea	Creatinine	Hemoglobin grams	Red blood cells mil-lions	Blood pressure	
1	1	52♂	56	48	104		95	161	7.3	2.7	92/60	90/70
2	2	75♀	56	88			37	42	8.0	2.9	120/80	110/60
3	5	60♀	10	30			51	126	5.5	1.9	105/65	100/65
4	1	49♂	45	85	101	67	51	48	11.0	3.3	185/110	180/95
5	1	62♂	35	60	97	48	27	53	5.8	1.7	88/52	70/60
6	5	65♂	18	135	101	42	28	30	4.0	1.7	110/70	112/63
7	2	65♂		42			100		9.2	2.9	180/90	150/80
8	2	53♂		116			45	98	8.5	2.3	120/70	120/70
9	2	63♂		166	117	49	44	49	5.0	2.4	82/50	60/30
10	1	59♂	41	52					4.5	1.4	110/60	90/36
11	1	56♂	28	45	93	51	125	102	5.8	1.9	100/70	100/38
12	5	70♂	49	88		49	53	104	5.8	1.8	100/40	92/80
13	5	57♂	25	41		54	57	69	5.1	1.7	200/70	185/70
14	1	59♂	60	74	103	54	43	66	6.8	2.0	120/70	65/50
15	1	53♂	55	76			52		7.8	2.0	100/70	60/40
16	4	56♂	24	43			95		5.5	1.9	100/50	95/55
17	2	51♀	41	58			52		6.8	2.1	110/65	110/60

\* Day of admission after onset of hemorrhage.

† Maximum values during hospital course.

<sup>1</sup> Presented in abstract at the meeting of the American Society for Clinical Investigation, May 6, 1940, Atlantic City, New Jersey.

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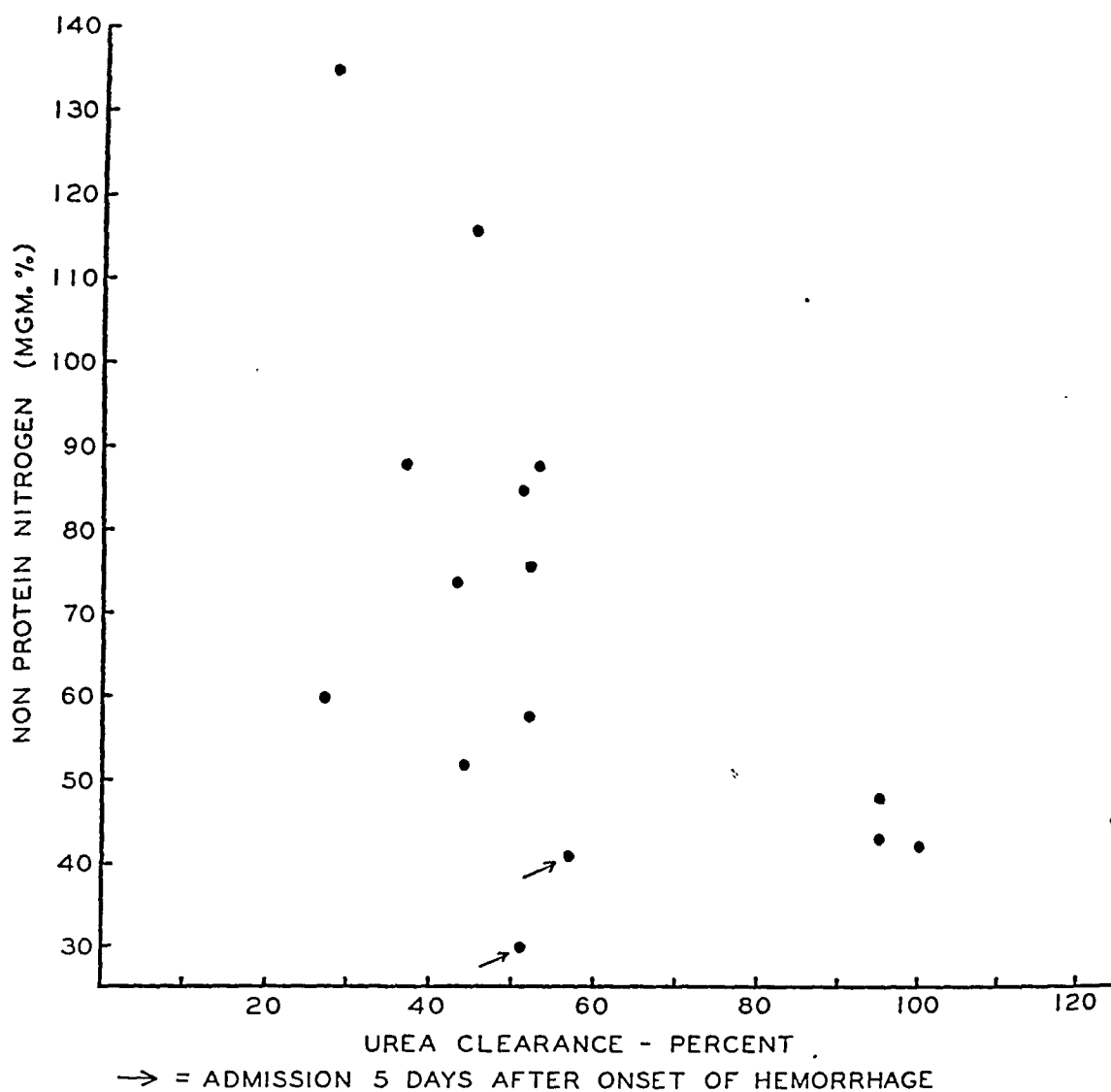


FIG. 1. COMPARISON OF BLOOD NONPROTEIN NITROGEN AND RENAL FUNCTION

function are listed. The azotemia which was observed in this group of patients occurred, for the most part, in the absence of shock.

Cases 1 and 11 are striking in that they entered the hospital on the day of onset, had severe hemorrhages, but showed no significant elevation of the blood nitrogen during the period of observation. Case 7 was similar, although his blood was examined the day after onset of hemorrhage. These 3 are the only cases in the series which showed consistently normal renal function. All the other cases entering the hospital prior to the third day after the onset of severe hemorrhage had a significant rise in the blood nonprotein nitrogen. Also, they had a marked reduction in renal function. Figure 1 is a comparison of the blood nonprotein nitrogen and the renal function. The correlation between the two is even more

striking when the course of this type of azotemia is considered.

The course of the blood nonprotein nitrogen in 6 representative cases is presented in Table II. In Cases 2 and 4 the clinical evidence was that of a single massive hemorrhage. Although these 2 patients developed a significant azotemia, the nonprotein nitrogen levels were again within normal limits of physiological variation on the fourth and fifth days after the hemorrhage had occurred. The course was more prolonged in Cases 14 and 15; in these patients there were repeated hemorrhages after admission to the hospital. Obviously, when there has been a single massive hemorrhage, a rise in the blood nonprotein nitrogen may be missed if the blood is not examined within the first 3 days after onset.

The hemorrhage was severe in all cases, but

TABLE II

*Course of blood nonprotein nitrogen after gastric hemorrhage*

Case	Percent urea clearance	Red blood cells	Days after onset of hemorrhage (Nonprotein nitrogen, mgm. per cent)											
			1	2	3	4	5	6	7	8	9	10	11	12
1	95	2.7	47	47									43	*
2	37	2.9	82	88										
4	51	3.3		85	75*	56	48				32	36*		
11	125	1.9		48	41			39	40	40			35	*
14	43	2.0		74		72	54	50	35	35		35		*
15	47	2.0		76	64	75		62	58		54	52		46

\* = First guaiac negative stool.

there was considerable variation in the maximum blood urea nitrogen levels. The lack of direct correlation between the severity of hemorrhage and the maximum blood urea nitrogen can be seen in Table I.

#### *Metabolism after hemorrhage*

Information concerning the protein metabolism incident to upper gastro-intestinal hemorrhage was obtained through the following experiment: Citrated human blood (1200 to 1500 cc.) was given to 4 patients by stomach tube within a period of 6 to 8 hours. These patients were given a constant nitrogen intake for 5 days preceding the administration of the blood. Their renal function was measured during the preceding 72 hours. The blood urea nitrogen rose in all cases as is usual after the ingestion of protein (11, 15, 20).

However, the rise was moderate and transient in the cases with normal renal function.

In Case 14, in whom the urea clearance was 50 per cent, the blood urea nitrogen reached 54 mgm. per cent and remained above 24 mgm. per cent for 72 hours. At the end of 24 hours the nitrogen (37.5 grams) derived from the 1250 cc. of blood ingested by this patient could be accounted for as follows: Nitrogen retained in the body water, estimated from a rise of 34 mgm. per cent in the blood nonprotein nitrogen, 15.5 grams; nitrogen excreted in the urine, 13.8 grams; extra nitrogen finally excreted in the feces, 8.7 grams; total nitrogen accounted for, 38 grams. Figure 2 gives a graphic illustration of this distribution. Nitrogen balance studies in the other 3 cases likewise indicated that the ingested blood was the source of protein responsible for the increase in urea production. Only 20 to 30 per cent of the nitrogen given as blood could be recovered in the stools when these were collected until the guaiac reaction became negative. The control stool nitrogen before the ingestion of blood and after the feces became free of blood was within normal limits and essentially the same. On the other hand, excess nitrogen amounting to 50 to 54 per cent of the nitrogen given as blood appeared in the urine. Results similar to those just noted were obtained in Case 14 when an equivalent amount of beef serum protein was ingested (see Figure 4). The effect of another type of protein was tested in another patient by

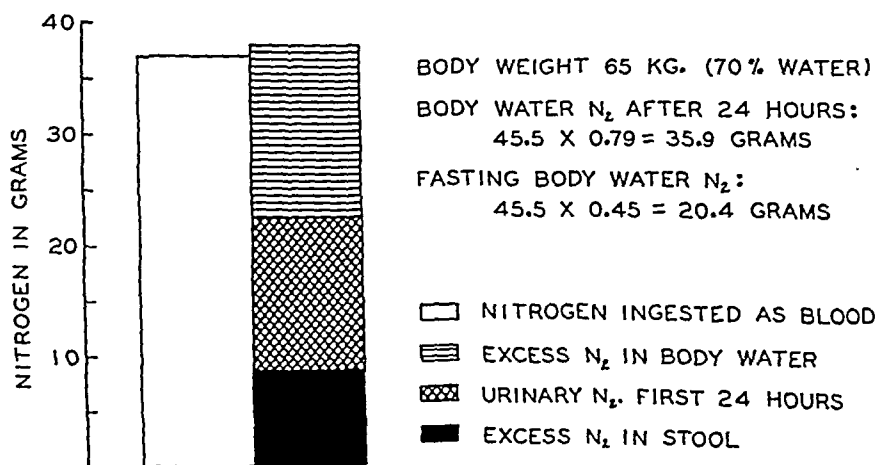


FIG. 2. DISTRIBUTION OF NITROGEN 24 HOURS AFTER INGESTION OF 1250 CC. OF BLOOD

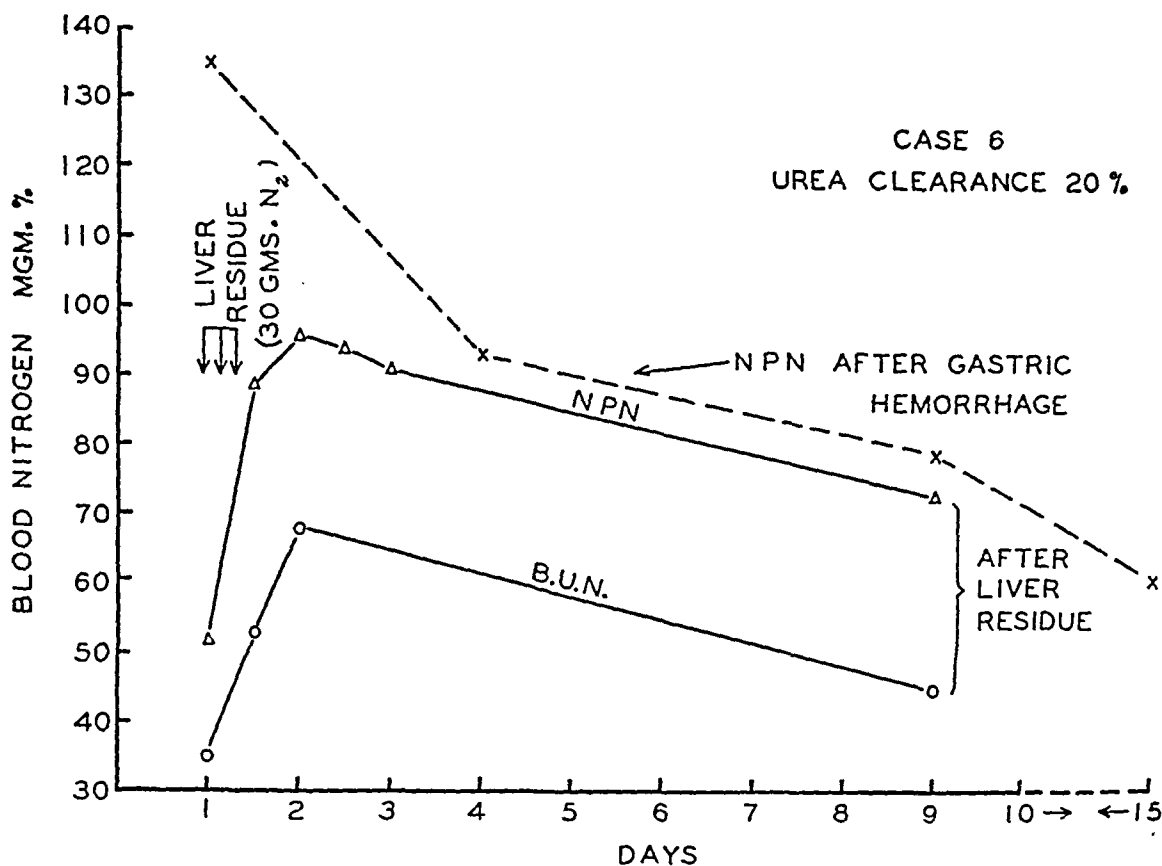


FIG. 3. COURSE OF BLOOD NONPROTEIN NITROGEN AFTER GASTRIC HEMORRHAGE AND AFTER INGESTION OF LIVER RESIDUE

N.P.N. = Nonprotein nitrogen

B.U.N. = Blood urea nitrogen

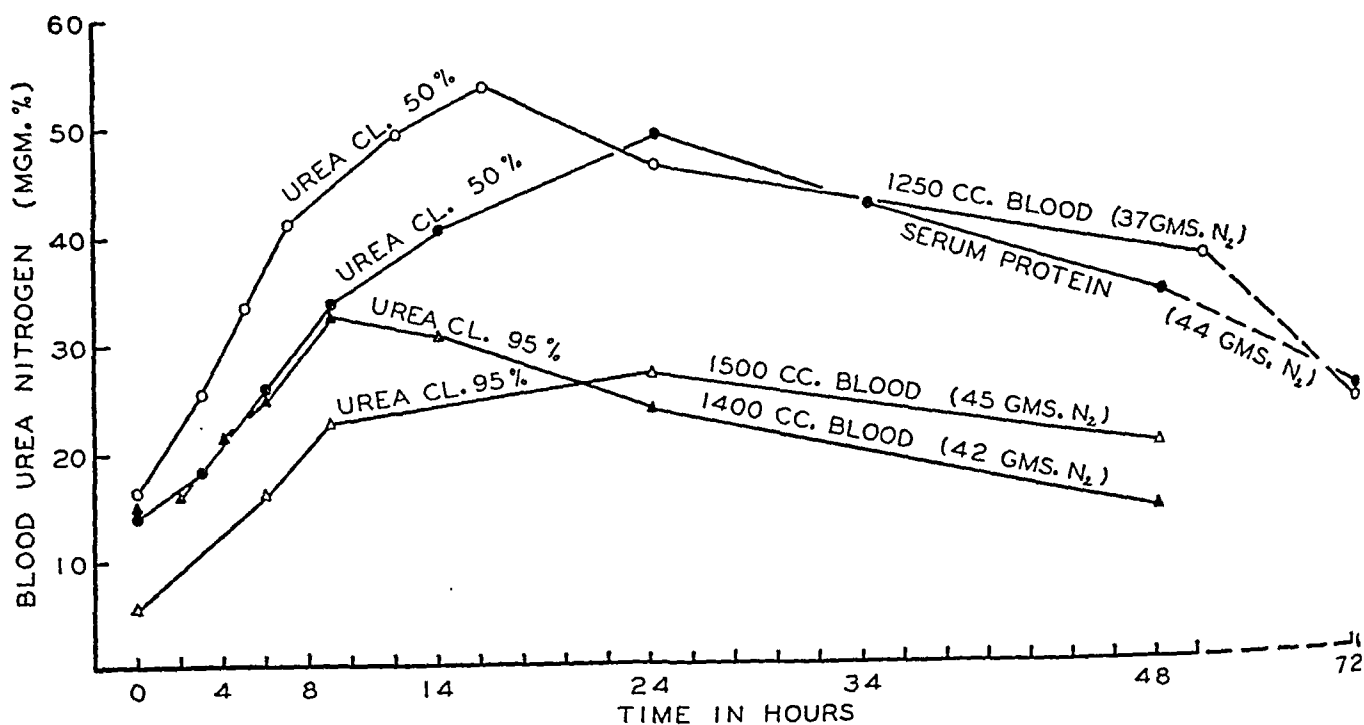


FIG. 4. ELEVATION OF BLOOD UREA NITROGEN AFTER INGESTION OF CITRATED HUMAN BLOOD AND BEEF SERUM PROTEIN

feeding liver residue. The behavior of the blood nonprotein nitrogen is shown in Figure 3, where it may be compared with the azotemia produced in this same individual by a massive spontaneous gastric hemorrhage.

An indication of the magnitude of the protein metabolism which takes place after gastric hemorrhage may be obtained from a study made on Case 14 shortly after admission to the hospital. During a period of 2 hours and 40 minutes, the urinary urea nitrogen output was 2.85 grams, at a time when the blood urea nitrogen was 50 mgm. per cent. If these conditions were continued for 12 hours, at least 214 grams of protein would be required for the production of the urea involved.

#### *Renal function after hemorrhage*

The data on renal function are shown in Table III. In most of the patients with reduced renal function, repeated measurement continued to show that impairment as long as 4 weeks after admission. The high incidence of impaired renal function was unexpected in that only 2 of these patients had positive histories and urinary findings of glomerulonephritis. It does not seem likely that the anemia was directly responsible for the impairment in view of the fact that those patients with normal renal function had equally severe anemia. In the cases in which the impairment of renal function was temporary, the fall in blood pressure was probably responsible.

As a whole, the patients with marked elevation in the nonprotein nitrogen recovered as rapidly as those with no significant elevation. The patients in whom the reduced renal function was incident to repeated fall in blood pressure had a more stormy course during the initial phase of their illness. No evidence was found to indicate that this type of azotemia represents a serious intoxication. However, the patients who ingested blood by stomach tube developed one or more loose stools consisting of partially digested blood within 7 hours after the feeding was started. Two of the patients became nauseated and vomited. A slight rise in temperature was noted in these latter cases.

The degree of azotemia was not a reliable index to the severity of the hemorrhage, but a progressive daily decline showed good correlation with

TABLE III

*Course of renal function in gastro-intestinal hemorrhage \**

Case number	Urea clearance, <i>per cent</i>				Creatinine clearance, <i>cc. per minute</i>			
	Week of hospital course							
	I	II	III	IV	I	II	III	IV
1	83 108		126 92		161		148	
2	35 39	55 52	37 41	58 54	42	58	63	67
3	54 48	78 70			126	120		
4	52 51	55 58	55 47		48	76	67	
5	29 25	66 70			53	103		
6	22 34	20 22			30	27		
9				72 72	49			114
10	46 42			74 73				138
11	129 121			137 90	192			167
12		53 54	66 59	65 50	104			118
13	62 52			65 53	60			87
14	44 42			49 51	67			67
15	52			56 40				74

\* Urea clearances done in duplicate. Creatinine and urea clearances done simultaneously. Normal creatinine clearance 170 cc. per minute (26).

the clinical evidence of cessation of hemorrhage. One patient in the group died (Case 10). He had repeated hemorrhages during the month prior to admission to the hospital. A final massive hemorrhage was responsible for his death.

#### DISCUSSION

In view of the fact that the elimination of urea from the body is effected primarily by the kidney, it seems reasonable that attention should be focused on renal function in the type of azotemia under consideration. The blood urea nitrogen

ranges from 5 to 23 mgm. per cent in the normal subject on a diet containing one gram of protein per kilo. body weight (15). From a physiological point of view the level of blood urea nitrogen is a function of urea production by the liver and urea excretion by the kidneys. It is conceivable that the production of urea may increase with such rapidity that it will exceed the excretory capacity of the kidneys and result in a rise in the blood urea nitrogen.

Evidence that the rate of urea formation is enormously increased when large amounts of blood enter the upper gastro-intestinal tract is to be found in our nitrogen balance experiments described above. In Case 14 the urinary urea nitrogen during the first 24 hours was 11.4 grams. The excess urea nitrogen in the body fluids at the end of 24 hours was 15.5 grams. This makes a total of 57 grams of urea formed during the 24 hours after the ingestion of blood. The urea production during this period is five times that of the control period in which the daily urine urea was 10 grams (4.8 grams urea nitrogen). If the renal function is reduced, either by a fall in blood pressure or by organic disease of the kidney, the elevation of blood urea nitrogen which follows the increased production of urea may be greatly accentuated.

The correlation between the elevation of blood urea nitrogen and the existing renal function in the cases presented here indicates that renal function is a most important factor in determining whether a given patient with a large gastric hemorrhage will develop a significant azotemia (see Figure 4).

Only a few reports on this subject are available in which adequate renal function studies have been recorded. The most recent paper is that of Stevens and co-authors (17), who studied kidney function in 4 cases. In each of their last 3 cases two or more of the urea clearance periods varied between 40 and 60 per cent of normal (corrected to 1.73 square meters surface area). Their first case was admitted to the hospital in shock (25). After a few hours the blood pressure rose from 80/60 to 122/60 and remained above that level during the remainder of the hospital stay. The urea clearances were done on the second hospital day, at which time they were within the normal range. Black (23) reported 12 cases of hema-

temesis in which renal function was studied. His data do not include the day after onset of hemorrhage on which the blood urea determination and urea clearances were made. That information is of much importance because of the transient nature of this type of azotemia. Even though his data are inadequate for analysis from our point of view, it is of considerable interest to note that the blood urea nitrogen did not exceed 20 mgm. per cent in any of his patients who had consistently normal renal function. Borst (6) reported 3 cases with azotemia in which the urea clearance fell to 15 per cent or less, associated with shock. In another paper this author reports 5 cases with azotemia (24). Three of these cases showed reduced renal function (19, 47, and 58 per cent of normal). The other 2 cases had normal renal function when determined several days after admission. Of these 2, Case 5 was admitted in shock with a blood pressure of 60/30. The urea clearance done on the third hospital day was normal. No statement concerning shock was made in his discussion of the other patient (Case 3). However, the patient received 700 cc. of blood by transfusion and considerable parenteral fluids during the first 48 hours. The urea clearance was done at some period during the second 24 hours. Alsted (12) reported 5 cases in which the renal function was studied. Four of these had normal renal function and the maximum blood urea nitrogen ranged from 23 mgm. per cent to 27 mgm. per cent. The other patient showed a fall in the urea clearance to 15 per cent with a rise in the blood urea nitrogen to 100 mgm. per cent. Clausen (7) reported 3 cases, all of whom showed a reduction in the urea clearance (38, 54, and 68 per cent). The data in Christiansen's cases (8) are too inadequate to allow definite conclusions.

It is seen from this brief review of the literature that a reduction in the urea clearance (10 to 60 per cent of normal) is a common finding in patients with a significant rise in the blood urea nitrogen following upper gastro-intestinal hemorrhage. Stevens and associates (17), in discussing the renal factor in this type of azotemia, point out that for the most part the urea clearances were not reduced below 20 per cent of normal. They cite Peters and Van Slyke to the effect that the urea clearance must be reduced to 20 per cent of normal before all cases of impaired renal function

show an elevated blood urea nitrogen. However, Peters and Van Slyke (18) also state that the blood urea nitrogen can be interpreted correctly only when considered in connection with the rate of urea formation. Indeed they point out that, if the protein intake is sufficiently reduced, many patients with extensive renal damage may continue to have a normal blood urea nitrogen. In the fasting state the urea clearance must be reduced at least to 20 per cent before the rate of urea formation exceeds the rate of urea excretion. When the rate of urea production is significantly increased, the rate of urea excretion will be exceeded with less severe reduction in the urea clearance.

Christiansen (8) suggested that a lack of blood chloride may be responsible for the azotemia of hemorrhage. That hypochloremia may result in azotemia seems established by the careful experiments in man by McCance (14). The rise in blood urea nitrogen which he observed was associated with a progressive decrease in renal function, although there was no change in blood pressure. None of the cases in our series in which the blood chloride was determined, showed hypochloremia (see Table I). No change was found in the blood chloride of Case 14 after the ingestion of blood. The plasma carbon dioxide combining power was not abnormal in this group of cases.

#### CONCLUSION

1. A marked rise in the nonprotein and urea nitrogen of the blood was observed in cases of severe upper gastro-intestinal hemorrhage only when there was a temporary or permanent reduction in renal function.

2. The duration of the azotemia was 3 to 5 days after a single hemorrhage, but was more prolonged when there were repeated hemorrhages.

3. No changes were observed in the serum chloride or in the plasma carbon dioxide combining power.

4. Whole blood in the upper gastro-intestinal tract is digested and absorbed, and results in the formation of urea.

5. The pathogenesis of the azotemia depends on a rate of urea formation which is in excess of the rate at which urea can be excreted by the kidneys.

6. No definite correlation between the degree

of azotemia and the prognosis for recovery was found.

7. The absence of azotemia in some patients with massive hemorrhage into the upper gastro-intestinal tract may be explained by the fact that these patients have normal renal function. In them the rate at which urea is excreted quickly equals the rate of increased urea production.

Grateful appreciation is expressed to Dr. Samuel H. Bassett for many valuable criticisms and suggestions during the course of this work.

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# ON THE RENAL TUBULAR EXCRETION OF CREATININE<sup>1</sup> IN NORMAL MAN

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The demonstration that in man the renal excretion of exogenous creatinine<sup>1</sup> takes place in part by an active tubular process depends upon two experimental findings (1). The plasma clearance of creatinine in the normal individual is invariably in excess of the simultaneously determined inulin clearance, which is accepted as a valid measure of glomerular filtration rate (2). Furthermore, raising the plasma creatinine from low to high concentrations depresses its clearance toward that of inulin, which appears to be its limiting value. However, contrary to similar findings in the dog-fish (3) and chicken (4), this depression is not completely reversible.

More recently, both the presence and the significance of the curvilinear relationship between plasma concentration and renal excretion of creatinine have been questioned. Winkler and Parra (5) observed that, following the ingestion of a single dose of creatinine, its clearance and the creatinine/sucrose clearance ratio behave rather erratically, but are generally depressed as the experiment progresses. These authors believe that the curvilinear relationship, as previously described, is not a dependent one. They suggest that its presence in the original experiments was incidental to an experimental routine which usually resulted in the high plasma concentrations being observed later than the low ones. Findley (6) later re-examined the relationship between plasma concentration and renal excretion over a range of plasma values of 1.0 to 14.0 mgm. per cent. His findings are precisely the same as those previously reported by Cope (7). That is, a linear relationship between these two variables obtains if one arbitrarily corrects the observed plasma concentration by —0.5 mgm. per cent of assumed non-creatinine chromogenic material. He did not consider the depression of the creatinine

clearance at higher plasma levels valid evidence of tubular excretion, since the concentrations necessary to demonstrate the phenomenon were "far beyond the physiological range." The linear relationship limited to the lower plasma values was then advanced as evidence opposing the renal tubular excretion of creatinine. This conclusion would favor Rehberg's (8) original contention that creatinine is excreted solely by glomerular filtration.

More recently, Rehberg (9) has called attention to the demonstration by Abdon (10) that the administration of creatinine leads to the appearance in the plasma of a substance resembling creatinophosphoric acid. It is Rehberg's belief that the tubules do not participate in the excretion of preformed creatinine but rather that they may transfer creatinine which has its origin in the plasma in some more complex compound, such as that described by Abdon. This is an important consideration, since this type of compound would yield true creatinine in ordinary plasma filtrates and behave as such toward both specific and non-specific analytical methods.

## EXPERIMENTAL METHODS

The subjects were healthy males selected from the wards of the Third (New York University) Medical Division of Bellevue Hospital. They had been admitted for minor illnesses from which they had completely recovered at the time these observations were made. In general, conditions were quasi-basal. The patients were at complete bed rest the morning of the experiment and had not received food for 16 hours. They were hydrated by the administration of 1000 ml. of water, 120 and/or 60 minutes prior to the first period. In long experiments a high urine flow was sustained by the administration of 250 to 500 ml. of water between groups of observations.

All urine collections were by catheter with completeness of collection insured by bladder washouts. The blood samples were obtained by puncture of the antecubital vein at the middle of each experimental period. The samples were centrifuged at once and the plasma precipitated as soon as separation was effected. Heparin was used as the anticoagulant. The urine samples were

<sup>1</sup> In this report, unless specifically stated to the contrary, the term creatinine may be taken to mean exogenous creatinine.



diluted to the expected U/P ratio of inulin and this diluted urine subjected to the same precipitation reagents as the plasma. The routine of analyses was much the same as in our previous work (1, 2), except that for inulin determinations the Folin (11) sugar method was used. All analyses were in duplicate and the mean of these was used in the calculation of the clearances.

Three general types of experiments were performed. In all, the plasma concentration of inulin was elevated to and maintained at approximately 100 mgm. per cent by appropriate priming and sustaining infusions. When creatinine was given intravenously, it was included in the inulin infusion, or it was injected into the lumen of the infusion tubing when a single intravenous injection was given. When given by mouth, the creatinine was dissolved in water, chilled and made palatable by the juice of a lemon. The details of experimental procedure and typical plasma concentrations can be obtained by reference to Tables I, II, and III.

In three experiments we examined the plasma for the presence of a labile combination of creatinine or derivative with phosphate following the administration of creatinine. After a control sample of blood, 10 grams of creatinine were administered by mouth and further blood samples taken at 1, 2, and 3 hours. The samples were handled in a cold room,  $+1.0$ — $+2.0^{\circ}$  C., with all apparatus and reagents cooled to this temperature by a stay overnight at the low temperature. The bloods were centrifuged at high speed for 2 minutes and the plasma separated and precipitated immediately with trichloroacetic acid. The filtrates, obtained with the aid of a vacuum pump, were then neutralized to pH 7.5 to 8.0 with NaOH. The entire operation, *i.e.*, vein puncture to the neutralization of the filtrate, required less than 5 minutes. The low temperature obviated the necessity of an anticoagulant. At the end of the experiment the neutralized filtrates were brought to room temperature and the method of Fiske and Subbarow (12) was used to determine the presence of a labile phosphoric acid compound. The rate of color development in this method was determined by readings each minute on an Evelyn photoelectric colorimeter. This rate was precisely the same in the test samples as in the control, and not significantly different from standard phosphate solutions which had their salt content enriched by dilution with trichloroacetic acid, neutralization with NaOH and subsequent acidification.

#### EXPERIMENTAL RESULTS

*On the depression of the creatinine clearance by simple elevation of the plasma creatinine concentration.* Figure 1 presents the relationship between the plasma creatinine concentration and the creatinine/inulin clearance ratio in a series of seven experiments derived from our previous study (1). Each dot represents the mean ratio observed in three consecutive 10 to 20-minute

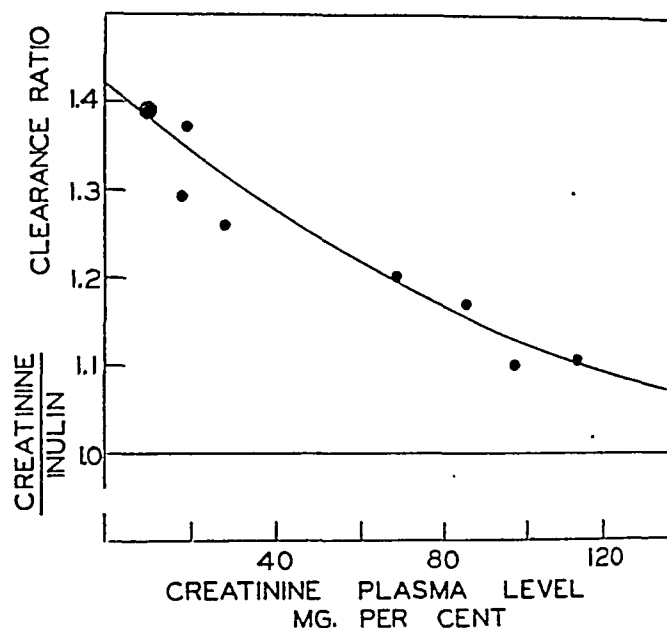


FIG. 1. THE RELATIONSHIP BETWEEN THE PLASMA CONCENTRATION OF CREATININE AND THE CREATININE/INULIN CLEARANCE RATIO

For full explanation, see text, p. 170.

periods started within 20 minutes after a single injection of creatinine; the circled dot is the mean of a large series of experiments at plasma concentrations varying from 5 to 15 mgm. per cent. The curve is constructed from the eighty observations in our original series of data and represents the mean of this relationship. These observations, for the most part, were from experiments which proceeded from low to high plasma concentrations by a series of two or three doses of creatinine. This comparison indicates that the creatinine clearance, and the creatinine/inulin clearance ratio, are dependently related to the plasma creatinine concentration as described by this curve. That is, the depression at the high plasma concentrations is a true expression of the relationship of the two variables.

*On the depression of the creatinine clearance related to the duration of observations at low plasma concentrations of creatinine.* Our data indicate that, after the oral administration of a single dose of creatinine, the initially elevated creatinine clearance may progressively fall. It should be stressed, however, that this phenomenon is wholly separable from that described in the previous section. We have attempted to define the circumstances of the fall experimentally and thereby gain some insight into the mechanism responsible for it. The experiments shown in

Tables I, II, and III are typical of the data as a whole. The lack of any systematic changes in the inulin clearances permits the presentation of our results in the form of creatinine/inulin clearance ratios or calculations derived from this term.

TABLE I

*An experiment wherein a constant intravenous infusion of creatinine was administered throughout*

Subject: P. F. Experiment number 8C.  
0-10 minutes. Priming infusion 100 ml. of 12.0 per cent inulin, 3.0 per cent creatinine in 0.85 per cent saline.  
10 minutes-end. Sustaining infusion, 4.5 per cent inulin, 0.6 per cent creatinine in 0.85 per cent saline at 4.0 to 4.5 ml. per minute.

Period	Concurrent time	Urine flow		Plasma concentration		Plasma clearance		Creatinine/inulin clearance ratio	Tubular activity	
		Inulin	Creatinine	Inulin	Creatinine	Inulin	Creatinine		C ratio-1.0	Fraction of control
	minutes	ml. per minute	mgm. per cent	mgm. per cent	ml. per minute	ml. per minute				mean
1	26.5-34	15.2	75.0	11.2	126	186	1.48	0.48	1.00	
2	-50.5	16.2	75.4	10.6	135	199	1.47	0.47		
3	-62	15.5	75.0	10.3	131	193	1.47	0.47		
4	117.5-128	15.2	83.0	11.4	133	187	1.41	0.41		
5	-139.5	14.9	86.8	11.7	124	176	1.42	0.42	0.83	
6	-152	15.7	90.9	12.4	136	182	1.34	0.34		
7	205.5-215.5	14.4	98.1	13.85	132	176	1.33	0.33		
8	-228.5	13.2	98.1	13.5	123	173	1.41	0.41		
9	-240	7.92	97.4	13.5	129	171	1.33	0.33	0.77	

TABLE II

*An experiment showing the fall in the creatinine clearance after a single oral dose of creatinine*

Subject: P. F. Experiment number 5C.  
0 minutes. 12 grams of creatinine by mouth.  
30-40 minutes. Priming infusion: 100 ml. of 12.0 per cent inulin in 0.85 per cent saline.  
40 minutes-end. Sustaining infusion: 4.5 per cent inulin infusion in 0.85 per cent saline at 4.0-4.5 ml. per minute.

Period	Concurrent time	Urine flow		Plasma concentration		Plasma clearance		Creatinine/inulin clearance ratio	Tubular activity	
		Inulin	Creatinine	Inulin	Creatinine	Inulin	Creatinine		C ratio-1.0	Fraction of control
	minutes	ml. per minute	mgm. per cent	mgm. per cent	ml. per minute	ml. per minute				mean
1	51-66	13.7	90.9	9.4	137.0	195	1.42	0.42	1.00	
2	-83	13.1	112.5	10.7	130.5	189	1.45	0.45		
3	-98.5	13.3	121.6	11.6	129.0	188	1.40	0.46		
4	148-162	15.8	125.0	10.5	141	179	1.27	0.27		
5	-179	13.2	125.6	10.0	129.3	163	1.26	0.26	0.60	
6	-193	9.09	125.6	9.5	129.0	161	1.25	0.25		
7	239-253	8.56	122.3	7.3	128.0	153	1.20	0.20		
8	-266.5	3.41	121.5	7.0	125.8	150	1.16	0.16		
9	-280	2.22	121.0	6.7	131.7	149	1.12	0.12	0.37	

TABLE III

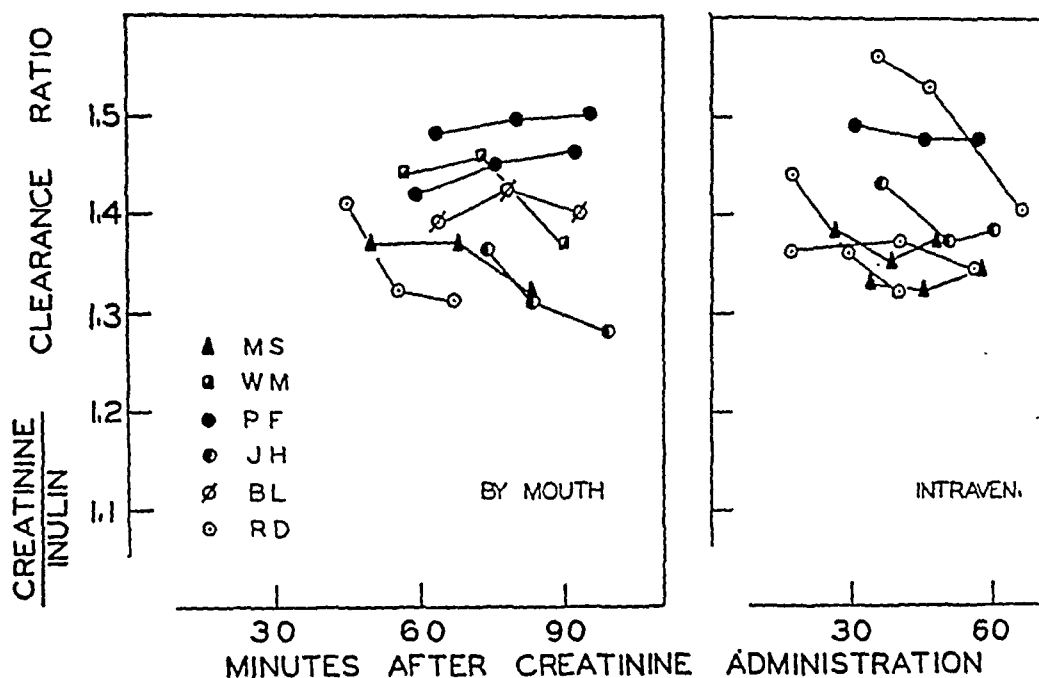
*An experiment showing the effect of a second small dose of creatinine on depressed creatinine clearances*

Subject: P. F. Experiment number 7C.  
0 minutes. 13.0 grams creatinine by mouth.  
290-300 minutes. Priming infusion: 100 ml. 12.0 per cent inulin in 0.85 per cent saline.  
300-371 minutes. Sustaining infusion: 4.5 per cent inulin in 0.85 per cent saline at 4.0-4.5 ml. per minute.  
371-381 minutes. Priming infusion: 100 ml. 4.5 per cent inulin, 3.0 per cent creatinine in 0.85 per cent saline.  
381 minutes-end. Sustaining infusion 4.5 per cent inulin, 0.8 per cent creatinine in 0.85 per cent saline at 4.0-4.5 ml. per minute.

Period	Concurrent time	Urine flow	Plasma concentration		Plasma clearance		Creatinine/inulin clearance ratio
			Inulin	Creatinine	Inulin	Creatinine	
	minutes	ml. per minute	mgm. per cent	mgm. per cent	ml. per minute	ml. per minute	
1	320-337.5	12.2	82.0	7.98	126	143	1.13
2	-342.5	12.2	82.1	7.31	129	151	1.17
3	-368.5	12.4	77.4	6.93	126	144	1.14
4	393-404	12.7	101.2	17.85	117	160	1.37
5	-417	13.6	95.6	16.80	125	167	1.34
6	-429	14.5	95.6	16.85	128	168	1.31

In Figures 2 and 3 we have plotted the individual clearance ratios of the initial periods which serve as controls in the experiments presented in Figures 4 and 5. These show the variations to be expected in the creatinine/inulin clearance ratio in three short consecutive periods. This is usually less than  $\pm 5.0$  per cent of the mean. The absolute values of the ratios have much the same distribution as those we have previously reported (1, 2). The data contained in these figures indicate that the mode of administration, i.e., intravenously or per os, is not a determining factor in the absolute magnitude of the initial ratios. The lack of systematic variation permits the use of the mean of the initial ratios as a standard of reference for the study of subsequent change in the system.

Typical experiments which examine the change in the tubular excretion of creatinine with time are given in Tables I and II; a graphical summary of all experiments in Figures 4 and 5. We have taken the creatinine/inulin clearance ratio — 1.0 as the measure of tubular activity in these figures. Each point is the mean of three experimental periods and is plotted as the fraction of the initial or control level of activity. Figure 4 shows that,



FIGS. 2 AND 3. CREATININE/INULIN CLEARANCE RATIOS OBSERVED SHORTLY AFTER THE ADMINISTRATION OF CREATININE

Each point is an experimental observation; the three periods in each experiment are connected by lines. In the experiments illustrated by Figure 2, a single dose of creatinine (10 to 13 grams) was administered by mouth; in those of Figure 3, a smaller amount (3.0 grams) was given intravenously and the plasma concentration maintained by a constant intravenous infusion. The plasma concentrations in both types of experiments were essentially the same.

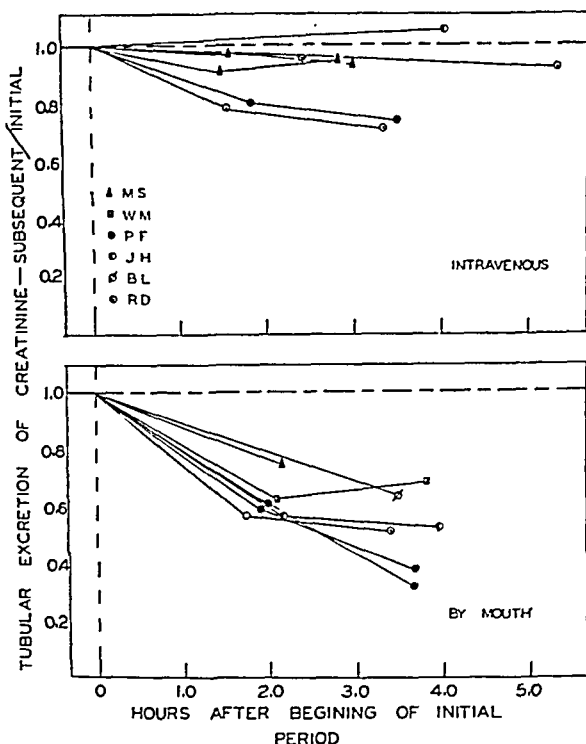
when the plasma creatinine concentration is maintained by a constant intravenous infusion, there may be no marked depression of tubular activity as the experiment progresses.<sup>2</sup> However, in those experiments where the creatinine was administered in a single oral dose, the depression, though variable, is quite definite. The detailed data indicate that the extent of this fall is not correlated with the absolute value of the initial ratio.

Our experiments do not reveal the maximal depression of tubular activity that may be expected, since practical considerations limit the duration of observation. Five hours after the oral administration of 13 grams of creatinine the plasma concentration in a normal man of average size is usually 5.0 to 7.0 mgm. per cent. At this time

we have observed ratios varying from 1.11 to 1.24 (see control ratios, Figure 6). Eight hours after a large single intravenous injection the ratio is in the same range (*cf.* 1, Figures 1 and 2). From this we are inclined to believe that, when there is a moderate concentration of creatinine in the blood, it is unlikely that the ratio will fall to 1.0; *i.e.*, that all tubular excretion of creatinine will cease.

After the creatinine/inulin clearance ratio has fallen, a second dose of creatinine usually elevates it to or towards the level characteristic of initial observations. Table III gives the details of one such experiment, while six are summarized graphically in Figure 6. It may be important that in two of the experiments the first period after creatinine seems significantly higher than subsequent ones; in these two it is actually higher than that observed initially in other experiments (see J. H., Figures 2, 3, 6). When there has been no depression in the ratio, a second dose of creatinine does not disturb the system. These negative experiments need no special presentation. The plasma concentrations in this group of experi-

<sup>2</sup> The errors inherent in the evaluation of this type of renal tubular activity are too great to permit a more precise description of the changes in activity we have observed. These figures (*i.e.*, 4 and 5) indicate first, a definite change in activity in the experiments where creatinine was given in a single oral dose, and secondly, that this change was greater than when creatinine was given by constant intravenous infusion. They do not permit the conclusion that no change takes place in the latter case.



FIGS. 4 AND 5. THE CHANGE IN TUBULAR EXCRETION OF CREATININE RELATED TO THE DURATION OF THE OBSERVATIONS

Figure 4 illustrates those experiments where the plasma concentration was maintained by a constant intravenous infusion of creatinine; Figure 5, those where a single dose of creatinine was administered by mouth.

Each point is the mean of a group of three short experimental periods which expresses the state of tubular activity at that time as the fraction of that observed initially. To better indicate the duration of the experiments, zero in the time scale is taken as the mid-point of the first period; the mean of the second group is plotted as the mid-point of the second group of periods, while the mean of the third group is plotted at the mid-point of the last period. The reader is referred to the text (p. 171) and to Tables I and II for further explanation.

ments are given in the legend of Figure 6. It will be noted that the plasma concentrations are sufficiently high to obviate any serious error from the endogenous chromogenic material.

*On the presence of a phosphocreatine-like substance in the plasma after the administration of creatinine in man.* In three experiments we have examined the plasma for the presence or absence of labile organic phosphate, 1, 2, and 3 hours after the oral administration of 10 grams of creatinine. In no sample was there evidence of

such a substance. Nor was there any systematic change in the concentration of plasma inorganic phosphate.

#### DISCUSSION

We reaffirm the curvilinear relationship between plasma creatinine concentration and its renal excretion and the independent nature of such evidence in demonstrating the active participation of the renal tubules in the excretion of this substance. Although such a relationship is not essential in theory, it has been demonstrated in every system of renal tubular excretion where adequate examination has been made. Furthermore, where

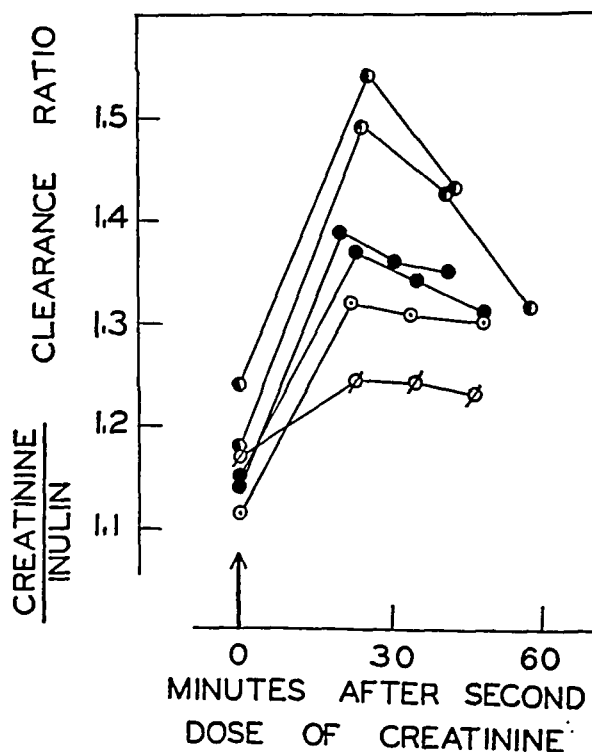


FIG. 6. THE EFFECT OF A SECOND DOSE OF CREATININE ON THE DEPRESSED CREATININE/INULIN CLEARANCE RATIO

The control values (zero time) are the means of three experimental periods obtained approximately 5 hours after the oral administration of 13 grams of creatinine. Subsequent points in each experiment are individual clearance ratios observed (at times indicated by the abscissa) after a small intravenous injection of creatinine. The change in plasma concentration (mgm. per cent) from the first group of periods to the second is given by the following mean values: P.F., 4.0 → 17.20; 8.60 → 20.6; B.L., 7.60 → 21.3; J.H., 4.9 → 16.0; 5.2 → 14.3; R.D., 6.9 → 15.3.

such a relationship has been described there is no evidence to controvert the presence of a process of tubular excretion (13). It is true that to demonstrate this relationship in the case of creatinine, plasma concentrations in excess of the physiological range are essential. However, the quantitative relationships which produce such a situation in no way diminish the forcefulness of the argument.

The origin of this curvilinear relationship is probably similar to that observed in other systems of renal tubular transfer; *i.e.*, it is due to an internal cellular limitation which manifests itself in a maximal rate of transfer (13). This possibility is somewhat strengthened by the observation that a maximal rate characterizes the tubular excretion of creatinine in the dogfish (14) and the chicken (4), and seems likely to be present in the aglomerular toadfish (15). In man, although the data are consonant with such a concept (*cf.* 1, Figure 5), the complications which serve as a basis for this report make its establishment and accurate evaluation difficult.

It is the process of tubular excretion itself, or the circumstances which may affect it, that must be examined for an explanation of the changes in the creatinine clearance not related to plasma concentration. Among the more obvious possibilities which may be responsible for this phenomenon are the following: (1) The mechanism of transfer might become "fatigued" incident to the continued presence of a high plasma creatinine. This impairment would express itself in an inability of the tubular mechanism to continue the transfer of creatinine with the same facility as was observed initially. (2) A process of adaptation or accommodation to the high plasma concentration might also produce the same result. In this view, essentially that of Miller and Winkler (18), little or no activity of the mechanism at endogenous plasma concentrations would be replaced for a time by a relatively high degree of activity on the presentation of a sudden increment in plasma concentration; this activity subsequently diminishing with simple maintenance of the elevated level. (3) The nature of the circulating creatinine might differ at endogenous levels, immediately after administration and sometime later. In this view, the renal mechanism itself would not be responsible for the apparent change in its activity.

The expression "fatigue" is used in the chemico-physiological sense. That is, accompanying activity of a system there occurs an expenditure or dissipation of some prerequisite for normal activity, or the accumulation of some substance as the result of activity which in itself impairs the subsequent working of the system. Physiological properties such as accommodation or adaptation are less easy to define in terms that would have any meaning in relation to a mechanism responsible for the tubular excretion of a substance. However, in the systems examined up to the present time, including those responsible for the transfer of creatinine in the aglomerular toadfish, dogfish and chicken, there is no evidence to indicate the presence of any of these properties. Nor do our present results indicate that they are contained in the system under examination. The restoration of the depressed tubular excretion toward the initial level by a second dose of creatinine, and the maintenance of essentially full activity<sup>2</sup> in the experiments where the plasma concentration was maintained by constant intravenous infusion seem to dismiss these possibilities.

Our results would be consonant with the viewpoint that a derivative of creatinine is formed subsequent to its administration to man. It is presumed that such a substance would react in plasma filtrates to ordinary chemical methods as does creatinine itself, but would be handled less efficiently by the mechanism of tubular transfer than the parent substance.<sup>3</sup> This type of change would be quantitatively more important in those experiments where a single oral dose is given than in those where the plasma concentration is sus-

<sup>3</sup> It is impossible to state how large a portion of the creatinine would have to undergo the change we suggest. If the material is simply handled less efficiently by the tubules, or not at all, it would be expected in the plasma in proportion to the lowering of tubular excretion; *i.e.*, 3 hours after creatinine by mouth it would make up a minimum of 50 per cent of the total analyzed concentration. On the other hand, it might act in a wholly different fashion. In terms of our conception of the cellular limitations in such systems of transfer (*cf.*, 12, p. 81), it could have a high affinity for the cellular element of the system and a slow rate of dissociation in the second reaction. In this case, minimal plasma concentrations would have a marked depressing effect upon tubular excretion. If the latter is the case, it poses an almost unsolvable problem, since material producing this effect need not have the chemical properties of creatinine.

tained by a constant intravenous infusion. In the former the creatinine would be exposed to this change for a longer duration of time.

The indirect nature of our evidence permits no more than a tentative suggestion that this may be the case. It is true that the body is capable of performing chemical operations on creatinine as evidenced by its incomplete recovery after administration (16). This experimental fact acquires added significance from the demonstration that this loss is not due to simple hydration to creatine (17). But there is no evidence that this loss is connected with the formation of a substance which conditions the tubular excretion of creatinine. The type of compound described by Abdon (10), *i.e.*, a labile compound containing phosphoric acid appearing in the plasma subsequent to creatinine administration, would satisfy the requirements of our experiments. We have, however, been unable to confirm Abdon's findings.

There are two lines of evidence—one experimental, the other chemical—which are in conflict with our interpretation. These would place the site responsible for the changing tubular excretion locally in the tubular mechanism itself. Individuals with renal insufficiency severe enough to cause creatinine retention are stated to have, contrary to the normal, a high creatinine/inulin clearance ratio at endogenous plasma concentrations (18) and, subsequent to creatinine administration, to show no depression in creatinine clearance related to the duration of observation (19). The high ratios observed in this type of patient are not comparable to the normal, since the absolute rate of glomerular filtration has suffered a marked reduction. The lack of elevation in the ratio on the administration of creatinine seems the exception rather than the rule. The experimental data available do not justify the conclusion that, contrary to the normal, after the administration of creatinine no change in tubular excretion takes place which may be related to the duration of the experiment. In eight of the twelve experiments which are purported to demonstrate this (19), there was a fall in the absolute value of the creatinine clearance. Although the creatinine/sucrose clearance ratios were more or less constant in those where it was observed, the sucrose clearances were far too erratic to permit their use as an adequate standard of reference.

The second point of conflict stems from a careful study of the chemical nature of plasma creatinine based upon what seems to be a specific enzymatic method of analysis (20). This method does not disclose a significant amount of non-creatinine Jaffé-reacting material in plasma filtrates derived from normal man either before or after the administration of creatinine (18, 20). It may be that the study of rates of reaction with this method rather than simple destruction or non-destruction of chromogenic material would aid in the solution of this conflict.<sup>3</sup>

#### SUMMARY

The process responsible for the renal tubular excretion of creatinine in normal man has been re-examined. In order to isolate the tubular process for examination, glomerular filtration rate has been measured simultaneously with the observations on creatinine excretion.

1. We reaffirm the dependent curvilinear relationship between plasma concentration and the tubular excretion of this substance.
2. We confirm the fact that after the oral administration of a dose of creatinine (13 grams) the initially high clearance falls progressively with time.
3. If, after this fall, a second dose of creatinine is administered, tubular excretion is raised to or toward the initial level of activity.
4. When the plasma concentration is maintained by the constant intravenous infusion of creatinine, tubular excretion is usually maintained at a level close to that observed initially.
5. It is tentatively suggested that the phenomena described under 2 and 3 are not due to a change in the renal tubular mechanism itself, but rather that, subsequent to its entry into the body, some of the creatinine undergoes a change which makes it less readily transferable by the tubular mechanism.

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# STUDIES OF HEMOGLOBINEMIA AND HEMOGLOBINURIA PRODUCED IN MAN BY INTRAVENOUS INJECTION OF HEMOGLOBIN SOLUTIONS

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Hemoglobinuria is a striking feature of several hemolytic disorders, such as blackwater fever, the various paroxysmal hemoglobinurias, and certain types of acute hemolytic anemia. Available information indicates that hemoglobinuria in these conditions results from intravascular hemolysis with liberation of hemoglobin into the plasma.

Quantitative studies of the plasma and urine hemoglobin and of pigments derived from hemoglobin have been made recently in this laboratory on several patients with hemoglobinuria. It was thought that comparison of the findings in normal individuals in whom hemoglobinemia and hemoglobinuria were induced by intravenous injections of hemoglobin solutions, together with the findings in clinical cases showing hemoglobinuria, might clarify certain aspects of these clinical problems.

Sellards and Minot (1), Duesberg (2), and Ottenberg and Fox (3) have demonstrated that small amounts of stroma-free solutions of human hemoglobin may be injected in man without serious toxic reactions. The findings of these authors will be discussed below.

## METHODS

### *Preparation and injection of hemoglobin solutions.*

The hemoglobin solutions were prepared essentially as described by Sellards and Minot (1) and by Ottenberg and Fox (3). In some instances "bank" blood, discarded for intravenous use ten days after collection, was used as the source of hemoglobin without regard to the blood group; when this was not available, fresh blood was collected, using sodium citrate as the anticoagulant. The cells were washed three times with physiological saline, then laked with four volumes of fresh distilled water maintained at approximately 37° C. for one hour, with frequent shaking. An amount of freshly prepared 18 per cent saline solution equivalent to  $\frac{1}{20}$  of the volume of hemoglobin solution was added with resultant clouding, apparently due to aggregation of stromata. The solution was centrifuged for an hour and the clear supernatant fluid carefully removed and filtered through a Seitz filter to remove micro-organisms and remaining stromata (3);

then it was inspected for cloudiness or particulate matter and a culture in broth was made to ascertain its sterility. As noted previously (3), the solution, after centrifuging and before filtering through the Seitz filter, would repeatedly show further clouding on addition of a few more drops of the hypertonic saline solution. However, after filtration through the Seitz filter, clouding with additional saline no longer occurred. Passage through the Seitz filter was extremely slow; accordingly, when the larger volumes were prepared, the solutions were first filtered without sterilizing the apparatus, and the filter pad was changed as filtration became slowed. The entire amount was then passed again through a sterilized Seitz filter. The concentration of hemoglobin in the solution was measured by the Evelyn method.

From 20 cc. to 280 cc. of the solution were injected into an antecubital vein at the rate of approximately 20 to 30 cc. per minute. Before injection of the larger volumes of hemoglobin solutions the subject's urine was made alkaline by oral administration of sodium bicarbonate and was maintained alkaline throughout the period of hemoglobinuria. A glass of water was taken approximately every hour in addition to the fluid of meals. These precautions against possible kidney damage due to hemoglobin products are based on the observations of Baker and Dodds (4) and others.

*Chemical methods.* Venous blood was drawn as suggested by Ham (5) with a sterile syringe and needle which had been rinsed several times with sterile physiological saline solution. The blood was transferred to a tube containing a volume of 3 per cent sodium citrate solution equivalent to 10 per cent of the volume of blood, and gently mixed. The sample was centrifuged immediately and the plasma separated with care to avoid any contamination with red cells. The hematocrit value was measured on a blood sample using oxalate mixture as the anticoagulant (6). The dilution of the plasma by the citrate solution was calculated and the plasma hemoglobin and bilirubin values were corrected accordingly.

The concentration of hemoglobin in the plasma and urine was measured by the benzidine method essentially as described by Bing and Baker (7, 8). The stock hemoglobin solution used by Bing and Baker is not stable. A fresh hemoglobin standard was made daily by diluting 0.5 cc. of blood of normal hemoglobin concentration to 2000 cc. with distilled water. The hemoglobin concentration of the blood was measured with the Evelyn colorimeter (9). Control plasmas were diluted one to 10 with water and 1.0 cc. was taken for



## INTRAVENOUS INJECTIONS OF HEMOGLOBIN IN NORMAL SUBJECTS.

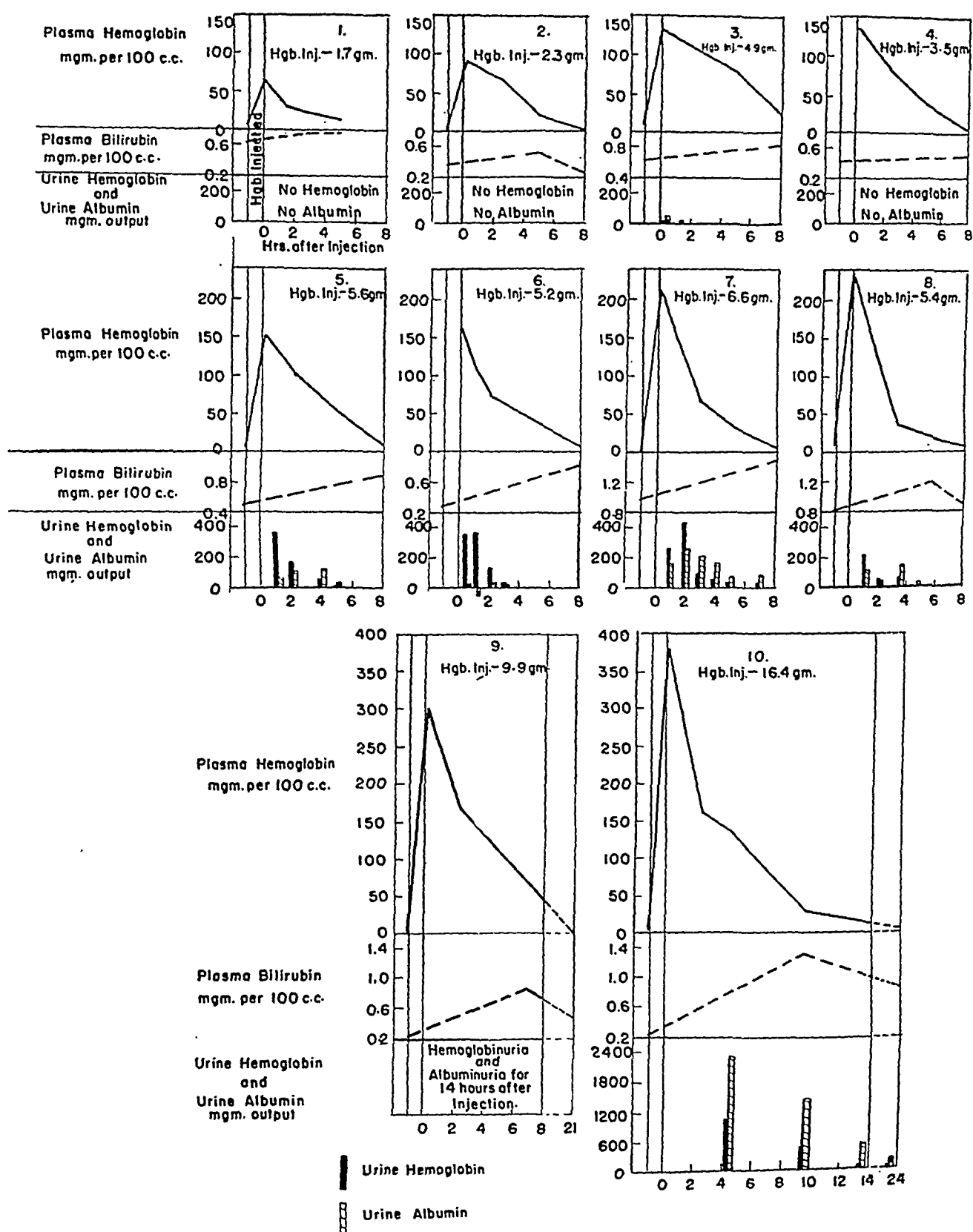


FIG. 1. THE HEMOGLOBIN AND BILIRUBIN CONCENTRATIONS OF THE PLASMA, AND THE HEMOGLOBIN AND ALBUMIN EXCRETION IN THE URINE FOLLOWING INTRAVENOUS INJECTIONS OF SOLUTIONS OF HEMOGLOBIN IN NORMAL SUBJECTS

The experiment number is given at the top of each diagram; each experiment was made in a different subject.

analysis. Appropriately smaller amounts of a one to 10 dilution were used for plasmas of moderate hemoglobin concentration. A one to 50 dilution was made of plasmas with 200 mgm. or more of hemoglobin per 100 cc. The hemoglobin standards were made from 0.1, 0.2, and 0.5 cc. of the standard hemoglobin solution so that the unknowns could be read in the colorimeter against a standard which closely matched. Samples of 0.1 cc. of undiluted urine, or 0.1 cc. of a diluted urine equivalent to approximately 5 mgm. per 100 cc., were used. This amount of an undiluted urine with high specific gravity precipitates some of the benzidine reagent and probably introduces a slight inaccuracy; if amounts of urine are chosen to read against the low standard, no precipitation occurs except in urines of very low hemoglobin concentration, which cannot be diluted. Appropriate amounts of water are added to the tubes containing 2 cc. of benzidine reagent to make the total of the unknown or standard hemoglobin samples plus water up to 1 cc.

The bilirubin concentration of the plasma was measured by the method of Malloy and Evelyn (10), using the Evelyn colorimeter. It was noted repeatedly that the bilirubin value obtained immediately after the hemoglobin injection was appreciably lower than that of the control blood, suggesting that the presence of hemoglobin interfered with the bilirubin measurement. Accordingly, hemoglobin solutions were added to normal plasma samples to give various final concentrations of hemoglobin and the effect on the bilirubin was measured. The bilirubin values were corrected for the 10 per cent dilution caused by the addition of the hemoglobin solution. The values obtained when the concentration of hemoglobin in the plasma was 30 mgm. per 100 cc., or over, were appreciably lower than those of the hemoglobin-free plasma (Table I). Consequently, the values for bilirubin obtained on plasma samples containing 30 mgm. of hemoglobin, or over, have been discarded except in one instance (Figure 1, Experiment 9).

The urea clearance was measured by the method of Van Slyke, Page, Hiller, and Kirk (11). The "urea plus ammonia" nitrogen was measured after urease action by the aeration method.

Urine "albumin" was measured quantitatively by determining the total amount of protein precipitated by trichloroacetic acid and subtracting from this the amount of hemoglobin as determined by the benzidine method. The method utilized to measure the total concentration of protein was as follows: In a weighed centrifuge tube the protein of 5 cc. of urine was precipitated with 5 cc. of 20 per cent trichloroacetic acid. The mixture was allowed to stand overnight and was then centrifuged at high speed. The supernatant fluid was decanted as completely as possible. Ten cc. of 10 per cent trichloroacetic acid was added to the precipitate, the mixture was stirred well and allowed to stand a few hours. The mixture was again centrifuged and the supernatant fluid was decanted. The tube was wiped clean on the outside, dried in the oven at 100° C. overnight and weighed after cooling in the desiccator. The weight of the precipitate

TABLE I

*Effect of hemolysis on plasma bilirubin values obtained by the Malloy and Evelyn method (10)*

Experiment number	Control plasma	Final hemoglobin concentration of plasma after adding hemoglobin			
		12 mgm. per 100 cc.	30 mgm. per 100 cc.	60 mgm. per 100 cc.	120 mgm. per 100 cc.
	Bilirubin	Bilirubin	Bilirubin	Bilirubin	Bilirubin
	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
1	0.69	0.69			
2	0.83	0.87			
3	0.18		0.17	0.21	
4	0.20		0.18	0.19	
5	0.49		0.35	0.31	
6	0.84		0.67	0.69	
7	0.92			0.63	0.58

was multiplied by 20 to give the concentration of total protein in 100 cc. of urine. Experiments with solutions of known hemoglobin concentration and of known albumin concentration gave satisfactory results with this simple method. Acetic acid and tungstic acid precipitations were tried with very unsatisfactory precipitation of protein in urines containing hemoglobin and very little albumin.

Spectroscopic examinations of plasma and urine were made with a Zeiss hand spectroscope. In one instance spectrophotometric examination of the urine was made with a König-Martens type of spectrophotometer.

## RESULTS

Fifteen intravenous injections of hemoglobin solutions, varying in volume from 20 to 280 cc. and in total hemoglobin content from 1.3 to 16.4 grams, were made. Ten injections were made in subjects who may be considered normal for the purposes of this study, four were made in patients with congestive heart failure and albuminuria, and one in a subject with carcinoma of the colon with metastases to the liver and a blood hemoglobin level of 40 per cent.

In twelve instances there were no febrile or other untoward reactions. One subject (Figure 2, Experiment 14) with bronchial asthma and congestive heart failure experienced transitory chills and fever after receiving 35 cc. of hemoglobin solution containing 1.9 grams of hemoglobin. In the two subjects (Figure 1, Experiments 9 and 10) in whom the largest amounts of hemoglobin were injected, *i.e.*, 10 grams and 16 grams, severe abdominal cramps with vomiting and visible peristalsis were experienced, coming

# INTRAVENOUS HEMOGLOBIN INJECTIONS IN SUBJECTS WITH CONGESTIVE HEART FAILURE AND ALBUMINURIA

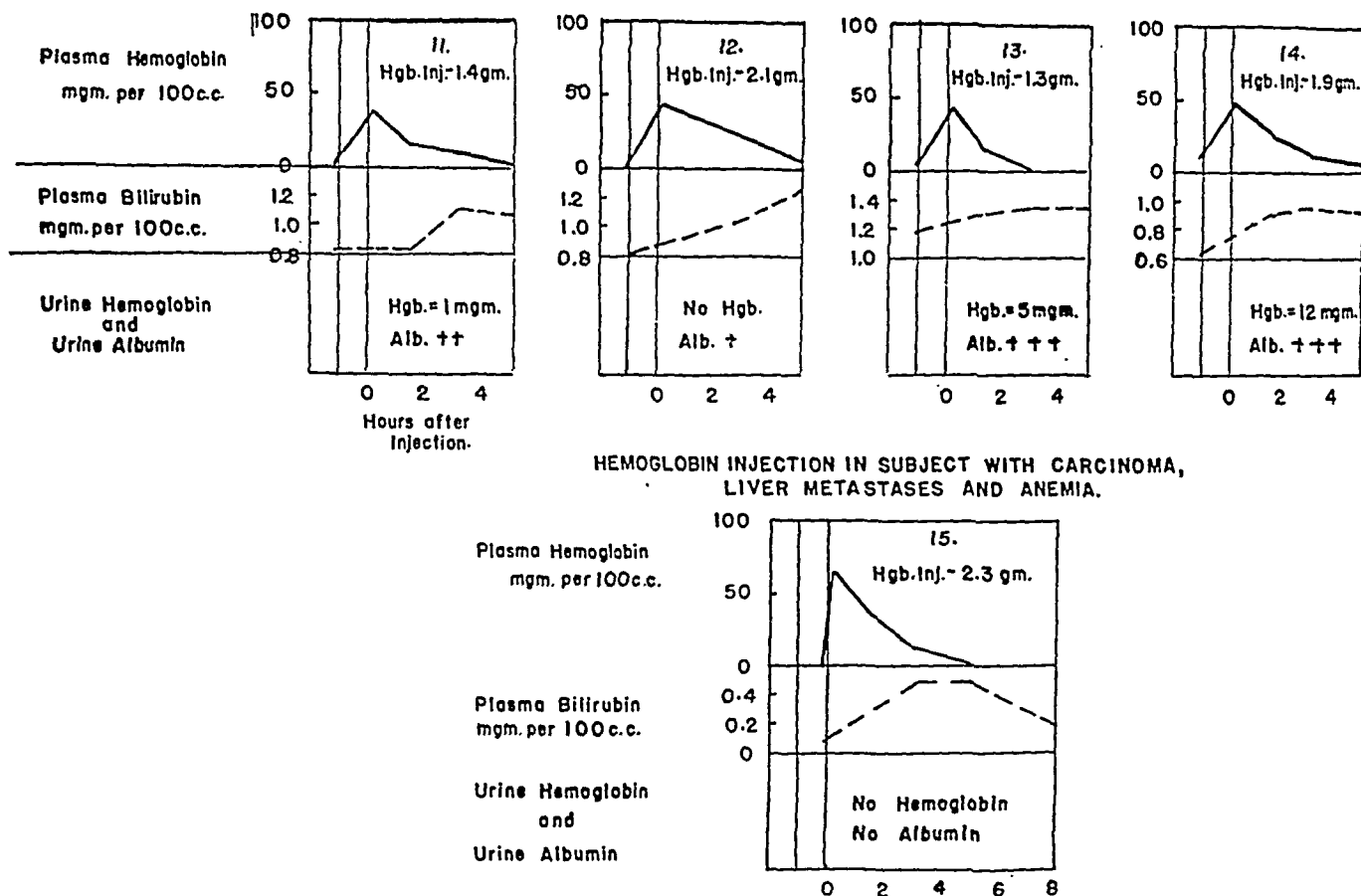


FIG. 2. THE HEMOGLOBIN AND BILIRUBIN CONCENTRATIONS OF THE PLASMA, AND THE HEMOGLOBIN AND ALBUMIN EXCRETION IN THE URINE FOLLOWING INTRAVENOUS INJECTIONS OF SOLUTIONS OF HEMOGLOBIN IN PATIENTS WITH CONGESTIVE HEART FAILURE, AND IN A CASE WITH CARCINOMA, LIVER METASTASES AND ANEMIA.

The experiment number is given at the top of each diagram; Experiments 11 and 12 were made in the same subject.

on about forty minutes after the injections and relieved immediately by calcium chloride given intravenously; the second subject, who received 16 grams of hemoglobin, had a moderate chill preceding the onset of the abdominal cramps, pains in the legs, and slight fever lasting for fifteen hours.

## Hemoglobinemia

The plasma hemoglobin was elevated by the injection of the hemoglobin solutions to values of 40 to 380 mgm. per 100 cc. (Figures 1 and 2). When levels of 40 to 60 mgm. of hemoglobin per 100 cc. were induced, approximately five hours were required for the plasma to be cleared of hemoglobin; with levels of 150 to 225 mgm. per 100 cc., about eight hours were required for clearing. In two studies in which levels of 280 and

380 mgm. of hemoglobin per 100 cc. of plasma were induced, the plasma hemoglobin did not reach normal for approximately twelve hours.

The curves representing removal of hemoglobin from the plasma show a more rapid rate of fall at the higher hemoglobin levels (Figures 1 and 2). Although there is considerable variation in different experiments, calculation of the average rate of drop at varying levels of plasma hemoglobin is of interest. The data of Ottenberg and Fox (3), as well as the data of this paper, have been utilized for this estimation (Table II); the averages of the two sets of data agree closely. Whereas the average rate of decline of plasma hemoglobin from 50 mgm. to 10 mgm. per 100 cc. is only 10 mgm. per 100 cc. per hour, the average rate of fall from approximately 250 to 150 mgm. per 100 cc. is 47 mgm. per 100 cc. per hour (Figure 3).

TABLE II

*Average rate of disappearance of hemoglobin from the plasma at various hemoglobin levels*

Author	Plasma hemoglobin		Average mid-point	Average rate of fall of plasma hemoglobin	Number of studies
	High level	Low level			
	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc. per hour	
Gilligan et al*.....	50	10	30	10	8
Ottenberg and Fox**..	50	10	30	10	5
Average.....			30	10	
Gilligan et al.....	132-150	50	98	29	8
Ottenberg and Fox....	150	50	100	27	7
Average.....			99	28	
Gilligan et al.....	212-295	150	202	49	4
Ottenberg and Fox....	202-288	150	201	46	8
Average.....			201	47	

\* Data of this paper.

\*\* Data of Ottenberg and Fox (3).

### *Hemoglobinuria*

In three normal subjects and in one patient with carcinoma and anemia in whom plasma levels of from 60 to 135 mgm. per 100 cc. were induced, no hemoglobin appeared in the urine (Figures 1 and 2). Hemoglobinuria occurred in seven cases in which the plasma hemoglobin levels after injection were 135 to 380 mgm. per 100 cc. The amounts of hemoglobin excreted in the urine were roughly proportional to the height of the plasma hemoglobin following injection. In Experiment 3, in which the plasma hemoglobin after injection was 135 mgm. per 100 cc., hemoglobin appeared only in the urine specimen voided one-half hour after injection and the total amount excreted was only 0.003 gram; in Experiment 10, in which the plasma hemoglobin was 380 mgm. per 100 cc. after injection, 1.6 grams of hemoglobin were excreted in the urine during the first ten hours after injection.

In three cases with congestive heart failure and albuminuria small amounts of hemoglobin were excreted after injections which produced plasma levels of only 38 to 49 mgm. of hemoglobin per 100 cc. (Figure 2). (The albumin concentrations in the urine before injection were 0.1 to 0.4 gram per cent.) The total amount of hemoglobin excreted was 0.001 gram to 0.012 gram. In one of these cases (Experiment 13) a distinctly red-

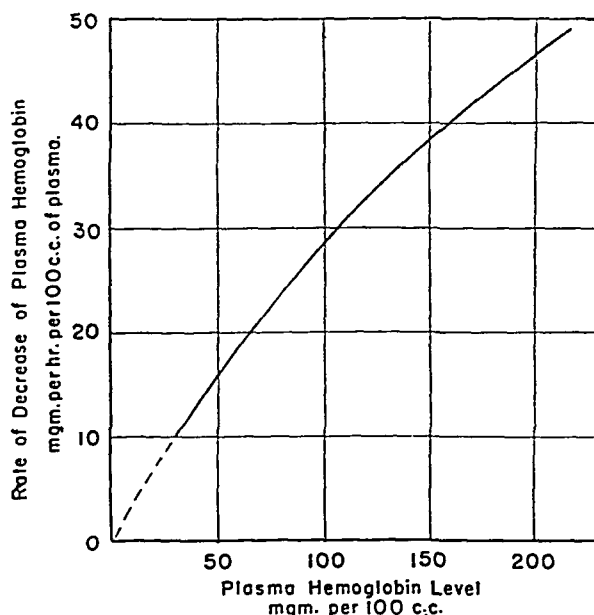


FIG. 3. AVERAGE RATE OF DECREASE OF THE HEMOGLOBIN CONCENTRATION OF THE PLASMA AT VARYING LEVELS OF PLASMA HEMOGLOBIN FOLLOWING INTRAVENOUS INJECTIONS OF HEMOGLOBIN SOLUTIONS

The data from which this curve has been constructed are given in Table II.

dish tint was present in small amounts of urine voided one-half, and one and one-quarter hours after injection. These urine specimens contained 16 and 11 mgm. of hemoglobin per 100 cc., respectively. In the two other cases the urine contained 3 and 5 mgm. of hemoglobin per 100 cc.; these specimens were not obviously discolored. In one of these patients a second injection of hemoglobin inducing a plasma level of 42 mgm. per 100 cc. failed to produce hemoglobinuria (Figure 2, Experiment 12); at this time cardiac decompensation was less severe and the urine albumin was only 0.02 gram per cent before injection.

Spectroscopic examination of fresh urines with the hand spectroscope revealed only oxyhemoglobin. No precipitated hemoglobin products were found. A spectrophotometric<sup>1</sup> examination of one fresh urine which was acid when voided revealed a point of maximum absorption at 576 mμ, which corresponds with the alpha band of oxyhemoglobin (12). In addition, there was a very

<sup>1</sup> This spectrophotometric examination was performed by Dr. John F. Taylor of the Department of Biological Chemistry of the Harvard Medical School.

faint point of absorption at 630  $m\mu$ , indicating the presence of an appreciable amount of methemoglobin (12).

### *Albuminuria*

Quantitative studies of the total protein of the urine were made as described above. When hemoglobin was present in the urine, the amount of hemoglobin as measured by the benzidine method was subtracted from the total amount of protein and the remaining protein was considered to be chiefly albumin. The positive identification of this protein as albumin was not accomplished because of the large amount of material required and the difficulty of the procedure of identification.

No albumin appeared in the urine of the normal subjects who received insufficient amounts of hemoglobin to cause hemoglobinuria. In normal subjects receiving amounts of hemoglobin sufficient to cause hemoglobinuria the "albumin" excretion was variable. In one subject (Experiment 6), in whom a total of 0.87 gram of hemoglobin was excreted in the urine during the first three hours after injection, there was no albuminuria, the total protein excretion being the same, within experimental error, as the hemoglobin excretion. In another case (Experiment 10), the total amount of protein was 6.2 grams, 1.6 grams of which were hemoglobin and 4.6 grams presumably albumin. In the other five normal subjects who showed hemoglobinuria (Figure 1), the total amount of "albumin" excreted was approximately equal to, or somewhat less than, the total amount of hemoglobin excreted. When "albumin" was present in the urine it usually continued to be excreted in concentrations of 10 to 50 mgm. per 100 cc. of urine for one-half to one hour after hemoglobinuria ceased.

### *Bilirubinemia*

The plasma bilirubin increased after injection of hemoglobin in every instance (Figures 1 and 2). In normal subjects, injections inducing plasma hemoglobin levels of 60 to 130 mgm. per 100 cc. caused an increase in bilirubin during the six to eight hours after injection of only 0.1 to 0.2 mgm. per 100 cc. of plasma over the control values. In studies in which the plasma hemoglobin was elevated to 150 to 230 mgm. per 100 cc., the bilirubin increased 0.4 to 0.5 mgm. per 100

cc. of plasma and, after two injections in which hemoglobin levels of 280 and 380 mgm. per 100 cc. were induced, the bilirubin increases amounted to 0.6 and 1.1 mgm. per 100 cc., respectively.

In the three subjects with congestive heart failure and in the patient with carcinoma and anemia, hemoglobin injections which induced plasma hemoglobin levels of only 40 to 60 mgm. per 100 cc. caused bilirubin increases of 0.2 to 0.4 mgm. per 100 cc. of plasma (Figure 2).

The plasma bilirubin appeared to return to the control value a few hours after the hemoglobin disappeared from the plasma in studies in which the smaller amounts of hemoglobin were injected (Figure 1). In Experiment 10 the plasma bilirubin rose from a control level of 0.2 mgm. to 1.3 mgm. per 100 cc. ten hours after the injection of 16 grams of hemoglobin. The plasma hemoglobin level ten hours after the injection was still elevated to 28 mgm. per 100 cc. so that, presumably, the bilirubin may have increased slightly further in the next two hours. Twenty-four hours after the injection the bilirubin level had fallen to 0.9 mgm. per 100 cc.

### *Kidney function*

Examination of the urinary sediment during periods of hemoglobinuria revealed nothing abnormal; no hemosiderin, hemoglobin casts or other precipitated hemoglobin products were found on examination of fresh urines. "Albuminuria," as mentioned above, was usually present during the period of hemoglobinuria and for approximately an hour thereafter.

None of the patients complained of pains in the back or burning on micturition.

The urea clearance was studied in one case (Experiment 7) in which injection of hemoglobin induced a plasma hemoglobin level of 212 mgm. per 100 cc. The fluid intake was maintained constant for several hours before and after the injection. The average urea clearance for three one-hour periods preceding the injection was 83 per cent of normal, and for the first three hours following injection, during which a total of 800 mgm. of hemoglobin was excreted in the urine, the average urea clearance was 76 per cent of normal. The average urine volume for the three hours before injection was 1.97 cc. per minute,

and for the three hours after injection, 1.82 cc. per minute.

### *Clinical observations*

The body temperature was not affected by the hemoglobin injections except in the two instances already described in which chills and fever occurred. There were no symptoms other than the untoward reactions already mentioned. The injections did not cause the spleen and liver to become palpable and produced no clinical icterus.

### DISCUSSION

The total amount of stroma-free hemoglobin solution administered in these studies was injected intravenously in from one to ten minutes; the results obviously would have been quite different if the same amounts of hemoglobin were administered by slow intravenous drip.

Toxic reactions occurred in three of the fifteen subjects. In two subjects abdominal cramps with vomiting and visible peristalsis, associated in one instance with transitory chills and fever, occurred following the injections of 10 grams and 16 grams of hemoglobin; these gastro-intestinal symptoms were relieved immediately by the injection intravenously of a solution of calcium chloride. The third subject experiencing a reaction had received only 2 grams of hemoglobin, following which transitory chills and fever occurred; this patient had a history of asthma and other allergic manifestations of thirty years' duration. Sellards and Minot (1) observed transitory chills and fever after the injection of approximately 10 grams of hemoglobin in solution in one normal subject. O'Shaughnessy (13) observed untoward reactions, namely chills and fever, pain in the back and loins, or sensations of constriction in the chest, following the intravenous injection of 10 to 50 grams of hemoglobin in solution in four patients with anemia. Fairley (14) observed nausea and fever in one subject after an intravenous injection of 21 grams of hemoglobin in solution and no untoward reactions in two subjects after injections of 14 and 17 grams of hemoglobin. The chills and fever, the pains in the back and loins, and the feeling of constriction in the chest experienced by some subjects after intravenous injections of 10 grams or more of hemoglobin in

solution are also noted in transfusion reactions and in other clinical hemolytic syndromes (15, 16) with or without hemoglobinemia. The immediate relief, following the intravenous administration of calcium chloride, of the severe abdominal pain, vomiting, and visible peristalsis which occurred in two subjects of this study suggests that these reactions were due to marked spasm of the smooth muscle of the gastro-intestinal tract. Similar abdominal crises occur in several clinical hemolytic syndromes (15, 16, 17). The laking of red blood cells releases appreciable amounts of a histamine-like substance (18) and of potassium (19), either one of which may cause constriction of smooth muscle.

Hemoglobinuria did not occur in the normal subjects in whom plasma hemoglobin levels of less than 135 mgm. per 100 cc. were induced but did occur in all instances when levels of 135 to 380 mgm. per 100 cc. were induced. Always less than 15 per cent of the injected hemoglobin was excreted in the urine. Sellards and Minot (1) observed hemoglobinuria in some normal subjects in whom amounts of hemoglobin presumably sufficient to induce plasma hemoglobin levels of approximately 150 to 300 mgm. per 100 cc. were injected, whereas hemoglobinuria did not occur after injections of smaller amounts of hemoglobin. Ottenberg and Fox (3) observed hemoglobinuria in four of eight studies of normal subjects with plasma hemoglobin concentrations of 110 to 150 mgm. per 100 cc. after injections of hemoglobin intravenously, and in nine of fifteen studies in which plasma levels of 175 to 320 mgm. per 100 cc. were induced. Thus, hemoglobinuria may occur in normal subjects when the plasma hemoglobin level is somewhat over 100 mgm. per 100 cc. but may be absent even at levels of almost 300 mgm. per 100 cc. Once hemoglobin has appeared in the urine, excretion continues in small amounts until the plasma hemoglobin level has decreased to 30 to 50 mgm. per 100 cc. (Figure 1).

The mechanism of the urinary excretion of hemoglobin is not entirely clear. Lichty, *et al* (20) conclude from experiments in dogs that hemoglobin passes the glomerular filter at plasma levels ("the renal threshold") considerably below those required to cause hemoglobinuria, but that the hemoglobin may fail to appear in the bladder urine because the filtered hemoglobin is rapidly

taken up and, in part at least, deposited within the tubular epithelium. When, however, the tubular cells become saturated with hemoglobin, hemoglobinuria occurs. On the basis of results of simultaneous creatinine and hemoglobin clearance studies in dogs, Monke and Yuile (21) have suggested that 3 per cent of the pores of the glomerular membrane are normally electrostatically large enough to permit the passage of an undissociated hemoglobin molecule.

Gersch (22) concluded that temporary renal injury, causing increased glomerular permeability and thereby allowing the filtration of hemoglobin, occurs in rabbits following intravenous injections of hemoglobin solutions. Hemoglobin, like serum albumin, has a molecular weight of 68,000.<sup>2</sup> Kerridge and Bayliss (23) found that Bence-Jones protein and other proteins with molecular weights of approximately 35,000 were freely excreted by the kidneys of various animals; "foreign" serum albumin with molecular weight of 68,000 was not excreted and hemoglobin with the same molecular weight was excreted in instances when the plasma hemoglobin level was high; "foreign" serum globulin with molecular weight of 104,000 and other proteins with still higher molecular weights were not excreted. These authors (23) conclude that proteins are excreted by the normal kidney according to the physical size of their molecules, but that hemoglobin has a peculiar place in this scheme. In our study small amounts of hemoglobin appeared in the urine at very low plasma hemoglobin levels after hemoglobin injections in the patients with preëxistent glomerular injury and albuminuria (Figure 2). Further, it was observed that protein<sup>3</sup> other than hemoglobin was

usually excreted in considerable amounts in the urine simultaneously with, and for a short time following, the excretion of hemoglobin (Figure 1), whereas no proteinuria was observed in the normal subjects who did not develop hemoglobinuria (Figure 1). These findings support the theory that hemoglobin appears in the urine only when the permeability of the glomerular membrane is increased. Several investigators have reported marked spasm of the renal arterioles after hemoglobin injections in animals (26, 27, 28); ephedrine, which also causes renal arteriolar spasm, may give rise to albuminuria (29). Microscopic examination of the urinary sediment revealed no abnormalities during hemoglobinuria and the urea clearance was not affected. There was no evidence that the hemoglobin injections in these studies produced any permanent kidney damage. Temporary or permanent renal insufficiency is observed, on the other hand, in several clinical syndromes associated with massive and prolonged hemoglobinuria (5, 30, 31).

As shown in Figure 3, removal of hemoglobin from the plasma after intravenous injection of this protein is more rapid at the higher concentrations of plasma hemoglobin. The rate of fall of plasma hemoglobin during periods of hemoglobinuria was dependent only to a small extent on the rate of removal by the kidneys; in Experiment 10, 10 grams of hemoglobin were removed from the plasma during the first five hours after the injection and only one gram appeared in the urine. The curve of Figure 3 affords a valuable basis of comparison by means of which quantitative plasma hemoglobin values obtained in patients with various hemolytic syndromes leading to hemoglobinemia may be utilized to yield information concerning the amount and rate of intravascular hemolysis occurring in these syndromes (30, 32).

After injections inducing plasma hemoglobin levels of 60 to 130 mgm. per 100 cc., the plasma bilirubin showed increases of only 0.1 to 0.2 mgm. per 100 cc. However, considerable increases occurred when hemoglobin levels of 150 mgm. per 100 cc., or greater, were induced by injections of 5 to 16 grams of hemoglobin, demonstrating that the rate of production of bilirubin exceeded the rate of excretion by the liver. The total amount of bilirubin produced by the breakdown of 5

<sup>2</sup> Whether hemoglobin may be dissociated into smaller molecules in the plasma is open to some question, since recent experiments indicate that dissociation may occur in dilute solution under certain circumstances (24, 25); it is presumed, however, that no dissociation occurs in the plasma, since studies in dogs have shown that below a certain plasma concentration hemoglobin does not filter through the glomeruli (20).

<sup>3</sup> Although the nature of this protein was not identified, the significance of its presence in the urine in relation to glomerular filtration would seem to be the same, whether the protein is albumin or a plasma protein derived from hemoglobin, since it did not appear in the urine in the absence of hemoglobinuria.

grams of hemoglobin would be approximately 200 mgm. That bilirubinemia should occur when this amount of hemoglobin is broken down in the course of four hours or so is expected, since in the bilirubin tolerance test in normal individuals the plasma bilirubin does not return to the control level for three or four hours after a single intravenous injection of 50 mgm. of bilirubin (33). Duesberg (2) and Fairley (14) have also observed small increases in plasma bilirubin lasting for six to twelve hours after intravenous injections of from 3 to 17 grams of hemoglobin in normal individuals; in cirrhosis of the liver and in cholelithiasis, the bilirubin increases were greater (2, 14). In our series, two patients with congestive heart failure and one patient with carcinoma with metastases to the liver and anemia showed bilirubin increases approximately twice as great as in normal individuals receiving the same amounts of hemoglobin. Our data and those of Duesberg (2) and Fairley (14) accord. Ottenberg and Fox (3) state that they observed no increase in plasma bilirubin following the injection of from 4 to 8 grams of hemoglobin in normal individuals. The data showing the degree to which the plasma bilirubin increases when varying levels of hemoglobinemia are induced in normal individuals (Figure 1) afford a basis for estimating the presence or absence of normal liver function in clinical attacks of hemoglobinemia.

Spectroscopic examination of the plasma and of the fresh urine in these cases revealed only oxyhemoglobin; a spectrophotometric examination of urine in one case showed mainly oxyhemoglobin and a small amount of methemoglobin. Methemalbumin, which is observed in the plasma of clinical cases with prolonged hemoglobinemia (14, 30), was not observed by spectroscopic or spectrophotometric examination of the plasma of our subjects or of Fairley's subjects (14) after hemoglobin injections. The plasma color was always light to darker red, with no brownish discoloration which occurs in the presence of appreciable amounts of methemalbumin (30). It has been shown in studies in animals that the character of the urinary hemoglobin pigments following intravenous injections of hemoglobin depends on the acidity of the urine (4, 34) and on the length of time which the urine remains in the bladder (20).

That the urine in our cases did not contain hemoglobin casts, acid hematin, or sufficient amounts of methemoglobin to be detected spectroscopically is ascribed to the circumstances that the urines were usually kept alkaline during the period of hemoglobinuria and that they were collected at frequent intervals and examined immediately.

#### SUMMARY

Fifteen intravenous injections of stroma-free human hemoglobin solutions were made in ten normal individuals, in three patients with albuminuria due to congestive heart failure and in one patient with carcinoma with metastases to the liver and anemia. The amounts of hemoglobin injected varied from 1.3 to 16.4 grams; the entire amounts were injected in from one to ten minutes.

There were no untoward reactions in twelve cases. Two subjects had chills and fever. One of these latter subjects and one additional subject had severe abdominal pains; these two subjects received the largest amounts of hemoglobin administered.

The plasma hemoglobin levels after injection varied from 40 mgm. per 100 cc. to 380 mgm. per 100 cc. The rate of decrease of plasma hemoglobin concentration was greater at higher hemoglobin levels. The average rate of decrease at varying levels has been plotted.

Hemoglobin was excreted in the urine in all the normal subjects in whom the plasma hemoglobin was elevated above 135 mgm. per 100 cc., and did not appear in the urine in normal subjects with plasma hemoglobin levels below 135 mgm. per 100 cc. Once hemoglobin had appeared in the urine, hemoglobinuria persisted until the plasma hemoglobin had decreased to as low as 30 to 50 mgm. per 100 cc.

Small amounts of hemoglobin were excreted in the urine of the patients with preëxisting albuminuria when the plasma hemoglobin level was elevated to only 40 to 50 mgm. per 100 cc.

In the normal subjects who did not show hemoglobinuria there was no proteinuria after injection. The concentration of the total protein of the urine during hemoglobinuria was usually greater than the concentration of hemoglobin, and proteinuria usually persisted for about an hour after cessation of hemoglobinuria. The excretion



of this additional protein, and perhaps the excretion of the hemoglobin itself, indicate temporary increased permeability of the glomerular capillaries in the subjects with hemoglobinuria. The urea clearance in one subject was the same before and during hemoglobinuria. The hemoglobin injections caused no permanent kidney damage. The mechanism of excretion of hemoglobin by the kidneys has been discussed.

The plasma bilirubin increased after the injection of hemoglobin solution in every instance. The degree of increase varied directly with the degree to which the plasma hemoglobin was increased by the injection. The amount of bilirubin increase after injection of a given amount of hemoglobin was somewhat greater in the patients with congestive heart failure and the patient with carcinoma with metastases to the liver and anemia than in the normal subjects.

The spleen and liver did not become palpable after the hemoglobin injections. Except in the two cases with chills and fever, the body temperature was unaffected.

Information concerning the pathologic physiology of certain clinical syndromes associated with hemoglobinemia can be obtained by comparison of the findings in cases with these syndromes with those of the present study.

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# OBSERVATIONS ON THE INHIBITION OF SULFONAMIDE ACTION BY PARA-AMINO BENZOIC ACID

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Considerable interest has been aroused recently in the investigation of substances which inhibit the action of the sulfonamide drugs. Lockwood (1) showed that peptones inhibit the action of sulfanilamide on streptococci. Stamp (2) isolated certain fractions of streptococci, and Green (3) isolated fractions of brucella, which have similar activity with respect to the corresponding organisms. West and Coburn (4) showed that under certain conditions coenzymes inhibit the action of sulfapyridine on staphylococci. MacLeod (5) found that the action of sulfapyridine on the colon bacillus in a synthetic medium can be nullified by extracts of certain animal tissues. Woods (6) extracted fractions of yeast which were capable of overcoming the bacteriostatic action of sulfanilamide on streptococci and on colon bacilli. From an analysis of the properties of the active extracts he was led to test a series of known chemical compounds and found that para-aminobenzoic acid had marked inhibitory properties *in vitro*. Selbie (7) supplemented this work by demonstrating that mice infected with streptococci could not be saved by sulfanilamide if para-aminobenzoic acid were administered at the same time. The reports of the two last named authors and the discussion of their work by Fildes (8) led us to undertake studies of some of the properties of para-aminobenzoic acid *in vitro* and in human subjects. In this paper are reported the results of observations on the following aspects of these studies: (1) the quantitative relationship between the bacteriostatic effect of sulfonamides on the pneumococcus and the inhibition of this effect by para-aminobenzoic acid; (2) the stages of the action of sulfapyridine on the pneumococcus during which inhibition by para-aminobenzoic acid is effective; (3) a consideration of the absorption and excretion of para-aminobenzoic acid and its chemical determination in blood and urine; (4) a comparison of the activity of the urine of human

subjects following ingestion of para-aminobenzoic acid with the activity of this drug when added *in vitro* to normal urine (the bacteriostatic effect of sulfathiazole on the colon bacillus was utilized for this purpose); (5) a study of the effect of oral administration of para-aminobenzoic acid on the bactericidal power of the blood of subjects receiving sulfonamides; and (6) the effect of para-aminobenzoic acid on rashes and fever due to sulfonamide toxicity.

## *Quantitative relationship between the concentration of sulfonamides and the inhibitory concentration of para-aminobenzoic acid*

The medium used in this and in the following experiment was beef infusion broth containing 0.05 per cent dextrose and 1 per cent bacto-peptone (Difco), to which was added 1 per cent defibrinated rabbit blood. Previous studies have demonstrated that in this medium sulfapyridine and sulfathiazole have definite bactericidal action on inocula of pneumococci up to several thousand per milliliter. The bactericidal action of sulfapyridine on pneumococci in this medium is about the same as in defibrinated blood (9). A stock type III pneumococcus (9) was used in this and in subsequent experiments. The virulence of this strain was maintained by mouse passage at weekly intervals. Both the para-aminobenzoic acid<sup>1</sup> and the sulfonamide drugs<sup>2</sup> (sulfanilamide, sulfapyridine and sulfathiazole) were dissolved in plain broth, sterilized by heating to 100° C. for 30 minutes, and made up in appropriate concentrations by dilution with broth. The pneumococci were grown in blood broth for 10 to 12 hours, diluted in plain broth, and 0.1 ml. of the appropriate dilution was added to 10 ml. amounts of the medium containing the test drugs to make a final concentration of about 50 viable diplococci per milliliter. The tubes were incubated at 37° C. and read at 24 and 48 hours for the presence of visible cloudiness indicating growth.

The results are charted in Figure 1. Within the range studied there was a roughly linear relationship between the concentrations of sul-

<sup>1</sup> Chemical grade, obtained from Eastman Kodak Company.

<sup>2</sup> Furnished by Lederle Laboratories, Inc.

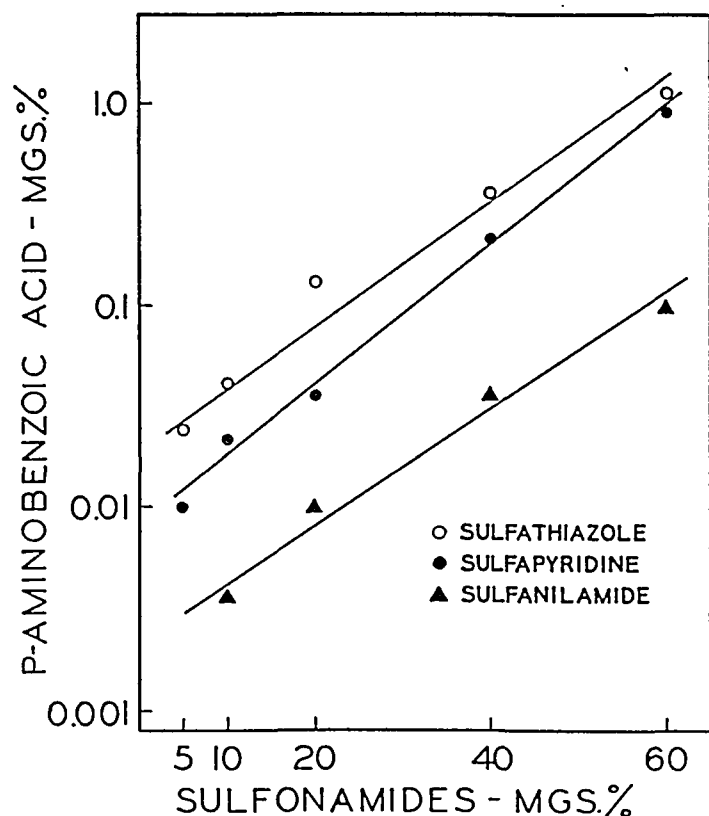


FIG. 1. MINIMUM CONCENTRATIONS OF *p*-AMINO-BENZOIC ACID REQUIRED TO INHIBIT THE BACTERIOSTATIC ACTION OF VARIOUS CONCENTRATIONS OF THREE SULFONAMIDE DRUGS

Semi-logarithmic scale.

fonamides and the minimum concentrations of para-aminobenzoic acid required to inhibit their bacteriostatic action. The inhibitory effect of para-aminobenzoic acid was most marked against sulfanilamide, less against sulfapyridine, and least against sulfathiazole. For example, the bacteriostatic action of 10 mgm. per cent of sulfathiazole under these conditions was inhibited by 0.04 mgm. per cent of *p*-aminobenzoic acid, whereas the action of similar concentrations of sulfapyridine and sulfanilamide was inhibited by 0.02 and 0.004 mgm. per cent, respectively, of para-aminobenzoic acid. These results can, of course, be interpreted in terms of the growth-stimulating effect of the various concentrations of the latter drug and the greater power of sulfathiazole to overcome this effect. Such an explanation was offered by Lockwood for the inhibitory action of peptone (1). The next experiment suggests that this interpretation is probably not justified.

### *The stages of sulfapyridine action during which para-aminobenzoic acid inhibition is effective*

It was shown in the preceding experiment that 1.0 mgm. per cent of *p*-aminobenzoic acid inhibited the action of sulfapyridine in concentrations up to 60 mgm. per cent. In the present experiment a number of identical tubes, each containing 10 mgm. of sulfapyridine per 100 milliliters of blood broth, were inoculated with 100 type III pneumococci per milliliter. Para-aminobenzoic acid sufficient to make a final concentration of 1.0 mgm. per cent was added to one tube at the beginning of the experiment. All the tubes were then incubated at 37° C. and blood agar pour plates were made at frequent intervals. Drug-free broth was used to make the proper dilutions of the explants. Two hours after the beginning of the experiment 1.0 mgm. per cent of *p*-aminobenzoic acid was added to a second tube. In like manner, equal amounts of this drug were added at 4, 8, 12, 16, 20 and 24 hours to the remaining tubes in succession. One tube, containing neither sulfapyridine nor *p*-aminobenzoic acid, and another, containing 10 mgm. per cent of sulfapyridine and no *p*-aminobenzoic acid, served as controls. Blood agar pour plate counts were made at frequent intervals.

The results are charted in Figure 2. The typical growth curve from a small inoculum of pneumococci in sulfapyridine broth is shown. There is an initial lag phase, a period of logarithmic increase, and then a phase of decline and death of the bacterial population (10). When *p*-aminobenzoic acid was added either initially, or during the lag phase or the phase of logarithmic increase, the resulting growth curve in each instance was the same as the normal control and there was no evidence of any sulfapyridine effect. When *p*-aminobenzoic acid was added during the static or declining phase of growth, however, there was a short lag period, following which the organisms began to grow rapidly again. It is evident that *p*-aminobenzoic acid can revive a culture in the presence of sulfapyridine at any stage in the growth curve as long as there are any viable organisms. In this case, at 20 hours there were only two diplococci per milliliter before the addition of *p*-aminobenzoic acid. The addition of the latter drug at 24 hours resulted in no growth, confirming the complete bactericidal action of the sulfapyridine at that time. The control curve of growth when neither sulfapyridine nor *p*-aminobenzoic acid was used was identical with the curve obtained with the latter drug alone. Under the

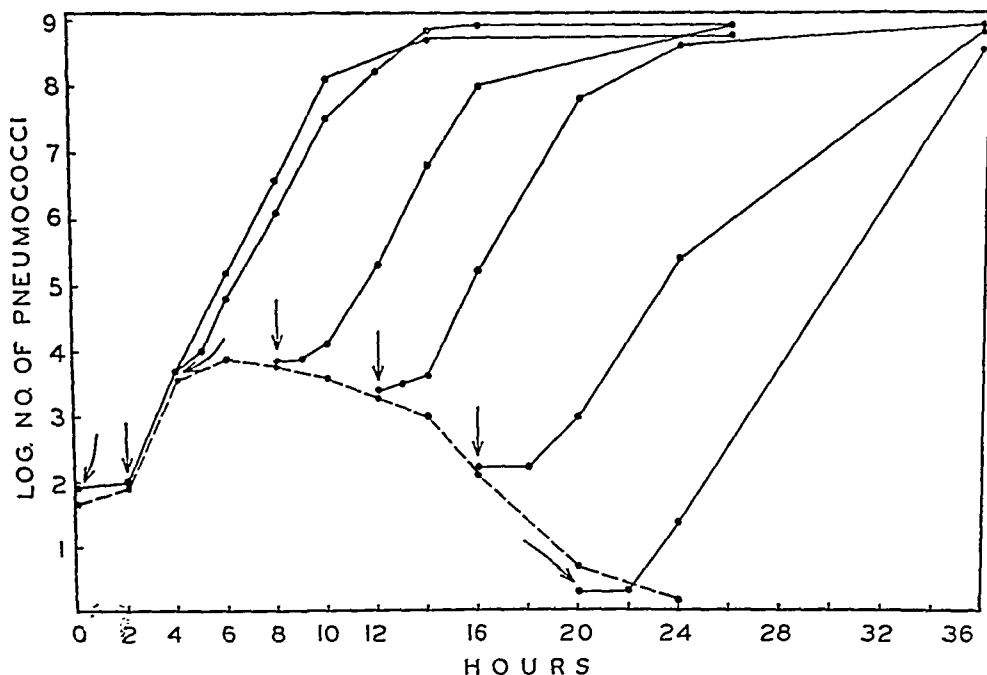


FIG. 2. EFFECT OF ADDITION OF 1.0 MG. PER CENT *p*-AMINOBENZOIC ACID TO THE GROWTH CURVE OF STOCK TYPE III PNEUMOCOCCUS IN BLOOD BROTH CONTAINING 10 MG. PER CENT SULFAPYRIDINE

Dotted line represents control curves of growth in sulfapyridine before addition of *p*-aminobenzoic acid at the various times indicated by the arrows. The curves representing growth when *p*-aminobenzoic acid was added at the beginning of the experiment and 2 hours later, and the control curve of growth in broth without the addition of *p*-aminobenzoic acid are all superimposed. The control sulfapyridine cultures were sterile at 24 hours and remained so even when *p*-aminobenzoic acid was added at that time.

conditions of the experiment, therefore, no additional growth-stimulating effect of *p*-aminobenzoic acid was apparent.

#### *Chemical determination, absorption, excretion and distribution of para-aminobenzoic acid*<sup>3</sup>

Para-aminobenzoic acid, being a primary arylamine, couples with *n*-(1 naphthyl) ethylenediamine dihydrochloride, producing a red color. It can therefore be detected in body fluids by Bratton and Marshall's method for sulfanilamide (11). However, when both *p*-aminobenzoic acid and a sulfonamide compound are present in blood or urine, it is impossible to estimate the quantity of each by this method.

Para-aminobenzoic acid is readily absorbed fol-

<sup>3</sup> We are indebted to Dr. F. H. L. Taylor for his advice and assistance in these studies.

TABLE I  
Blood levels of *p*-aminobenzoic acid in milligrams per 100 ml. following single oral doses

Subject	Dose	Hours after administration											
		1 hour		2 hours		3 hours		4 hours		5 hours		6 hours	
		F.	T.	F.	T.	F.	T.	F.	T.	F.	T.	F.	T.
M.C.	1.0	S.T.	0.8	0	S.T.			0	0	0	0	0	0
A.R.	2.0	1.5	2.2	S.T.	1.1			0	S.T.	0	0	0	0
T.L.	4.0	3.0	3.5	4.0	5.3	2.4	3.7			S.T.	1.0		
P.D.	4.0	6.1	6.8	5.0	6.1	2.6	3.9			S.T.	1.1	S.T.	0.9
L.M.	4.0	2.6	3.6	3.8	5.7	3.3	5.4			0.4	2.2		
J.E.	4.0			2.5	4.8								

S. T. = slight trace (less than 0.1 mgm. per 100 ml.).

F. = concentration of free *p*-aminobenzoic acid in milligrams per 100 ml., as determined by the method of Bratton and Marshall for sulfanilamide (11).

T. = total concentration of *p*-aminobenzoic acid in milligrams per 100 ml., as determined by the method of Bratton and Marshall for sulfanilamide (11).

lowing ingestion of from 1 to 4 grams (Table I). Maximum blood levels are reached in 1 to 2 hours. Conjugation of the drug apparently begins early and progresses rapidly. Four hours after doses of 1 or 2 grams the compound is no longer detected in the blood. After single 4-gram doses, small quantities are still present in the blood at 6 hours.

Urinary excretion is rapid and largely completed in 12 hours (Table II). About two-thirds of the drug is excreted in the "free" form. When determined as "total" drug (after acid hydrolysis), *p*-aminobenzoic acid is apparently completely recovered in the urine in 12 hours.

TABLE II

Urinary excretion of *p*-aminobenzoic acid following single oral doses

Subject	Dose	Hours after administration	Urine volume	F.	T.	Total excreted (cumulative)		Per cent of administered drug excreted (cumulative)	
						F.	T.	F.	T.
	grams		ml.	mgm. per 100 ml.	mgm.	mgm.	mgm.	per cent	per cent
M. C.	1.0	0-5	270	85	232	230	626	23	63
		5-11	700	0.9	5.0	236	661	24	66
A. R.	2.0	0-5	730	184	274	1340	2003	68	100
		5-11	390	20	24	1417	2097	71	105
T. L.	4.0	0-3	250	581	684	1453	1710	36	43
		3-6	360	322	534	2612	3632	65	91
		6-12	950	8.2	47	2690	4080	67	102
		12-24	1130	1.4	7.2	2705	4161	68	104
P. D.	4.0	0-3	340	557	710	1892	2413	47	60
		3-6	125	515	944	2535	3593	63	89
		6-12	475	24	90	2650	4021	66	101
		12-24	1100	2.0	9.3	2672	4123	67	103

F. = concentration of free *p*-aminobenzoic acid in milligrams per 100 ml., as determined by the method of Bratton and Marshall for sulfanilamide (11).

T. = total concentration of *p*-aminobenzoic acid in milligrams per 100 ml., as determined by the method of Bratton and Marshall for sulfanilamide (11).

Following absorption, this compound apparently enters the blood cells but is not equally distributed between cells and extracellular fluid. The concentration of *p*-aminobenzoic acid in blood plasma is about three times as great as its concentration in red cells (Table III).

TABLE III

Distribution of *p*-aminobenzoic acid between blood plasma and red blood cells

Subject	Concentration (mgm. per 100 ml.)				
	Whole blood	Plasma (P)	Cells (C) (Calculated)	C/P	Hematocrit (Corrected)
T. L. ....	Free 4.0	6.1	1.9	0.31	50.1
	Total 5.3	7.9	2.7	0.34	
P. D. ....	Free 5.0	6.3	2.3	0.37	31.7
	Total 6.1	7.9	2.2	0.28	

[ Blood obtained 2 hours after a single 4.0-gram oral dose.

*Inhibition by para-aminobenzoic acid of the bacteriostatic effect of sulfathiazole on the growth of B. coli in human urine. A comparison of urine obtained after ingestion of para-aminobenzoic acid with urine to which the drug is added in Vitro*

Urine was collected from a normal subject after oral administration of 4.0 grams of *p*-aminobenzoic acid, and the concentration of the compound excreted in the urine was determined colorimetrically. A specimen of drug-free urine was then obtained from another subject and *p*-aminobenzoic acid was added *in vitro* in an amount calculated to yield the same concentration of "free" drug as was present in the first sample. The two urines were filtered through Berkefeld candles and tested for sterility. They were then diluted with normal sterile urine to make concentrations of "free" *p*-aminobenzoic acid varying from 500 to 0.01 mgm. per cent and both series of dilutions were distributed into tubes containing 5 ml. each. Sulfathiazole in the form of a solution of the sodium salt in distilled water was added to each of the two series of *p*-aminobenzoic acid-containing tubes of urine. The final concentrations of sulfathiazole ranged from 500 mgm. per cent to 50 mgm. per cent. Concentrations of sulfathiazole smaller than 50 mgm. per cent were not regularly bactericidal and were therefore not used in this experiment. The final pH of the drug-containing urines varied from 7.0 to 7.8. A stock culture of *B. coli communis* was grown in broth and diluted in distilled water, and 0.1 ml. of the proper dilution, selected so as to make an inoculum of about 1500 viable organisms per milliliter, was added to each tube of urine. Visible clouding after 48 hours' incubation at 37° C. was used to indicate growth. At the end of 48 hours subcultures on agar were made to confirm the presence or absence of growth.

TABLE IV

*Pneumococcal activity of defibrinated human blood following administration of sulfonamides and p-aminobenzoic acid*

Experiment number	Time of blood samples in relation to drug administration	Growth inhibition		Pneumo-coccal action at 48 hours		Remarks
		24 hours	48 hours	Inoculum	Growth	
I	Control (before drugs)	0	0			Subject E. C. Single dose of 5.0 grams Na. SP. i.v., followed in $\frac{1}{2}$ hour by 2.0 grams p-ABA. p.o. Stock type III pneumococcus. Tests done immediately after last blood sample.
	$\frac{1}{2}$ hour after SP. (SP. level: F. = 10.5, T. = 10.5)	10 <sup>4</sup>	10 <sup>4</sup>	10 <sup>4</sup>	35	
	1 hour after p-ABA.	0	0			
	2 hours after p-ABA.	0	0			
	5 hours after p-ABA.	10 <sup>4</sup>	10 <sup>4</sup>	10 <sup>3</sup>	50	
II	Control (before drugs)	0	0			Subject J. E. Single dose of 5.0 grams Na. SP. i.v., followed in $\frac{1}{2}$ hour by 1.0 gram p-ABA. p.o. Stock type V pneumococcus. Tests done after incubation of blood samples at 56° C. for $\frac{1}{2}$ hour.
	$\frac{1}{2}$ hour after SP. (SP. level: F. = 10.2, T. = 10.4)	10 <sup>4</sup>	10 <sup>4</sup>	10 <sup>3</sup>	22	
	1 hour after p-ABA.	0	0			
	2 hours after p-ABA.	10 <sup>3</sup>	10	10	$\infty$	
	4 hours after p-ABA.	10 <sup>3</sup>	0			
III	Before first dose of p-ABA. (ST. level: F. = 4.9, T. = 5.2)	10 <sup>3</sup>	10 <sup>4</sup>			Subject S. L. Received ST. 1.0 gram every 4 hours. p-ABA., 2.0 grams every 3 hours, begun after fifth dose of ST., which was also continued. Stock type III pneumococcus. Tests done immediately after last blood sample.
	1 hour after second dose of p-ABA.	10	10			
IV	Before first dose of p-ABA. (ST. level: F. = 6.3, T. = 7.0)	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>4</sup>	10	Subject L. C. Received ST. 1.0 gram every 4 hours. On tenth day of ST. therapy received in addition 1.0 gram p-ABA. every 2 hours. Stock type III pneumococcus. Blood samples kept in icebox for 72 hours before testing.
	1 hour after fifth dose of p-ABA.	10	10	10	0	

*Growth inhibition.* Numbers represent the largest inoculum which showed no color change in the blood.

*Pneumococcal action.* Listed only when inhibition was noted. *Growth* refers to number of colonies grown in agar pour plates. *Inoculum* refers to original number added to the blood. Larger inocula yielded too many colonies to count; smaller ones yielded no growth.

*Abbreviations.* ST. = sulfathiazole

SP. = sulfapyridine

Na. SP. = sodium sulfapyridine

p-ABA. = p-aminobenzoic acid

i.v. = intravenous

p.o. = oral

F. = concentration of free sulfonamide in mgm. per 100 ml.

T. = concentration of total sulfonamide in mgm. per 100 ml.

∞ = innumerable.

This experiment was repeated several times. The general character of the results was similar in each instance and corresponded to the results obtained by Woods (6), who used sulfanilamide and a synthetic medium instead of sulfathiazole and urine, respectively. Quantitatively, however, the results varied widely, as may be expected with a medium as variable as urine. In each experiment there was a marked difference between the activity of p-aminobenzoic acid added *in vitro* and its activity when excreted in the urine. When added *in vitro*, 0.1 mgm. per cent of p-aminobenzoic acid usually inhibited the action of 250 mgm. per cent or less of sulfathiazole; when excreted in the urine, on the other hand, 10 mgm. per cent of p-aminobenzoic acid was required to inhibit the action of 100 mgm. per cent or less of sulfathiazole. The bactericidal effect of 500 mgm. per cent of sulfathiazole could not be overcome even by 500 mgm. per cent of p-aminobenzoic acid.

However, such high concentrations of p-aminobenzoic acid in themselves inhibited growth in control tubes containing no sulfathiazole. While these tests were not entirely satisfactory, they clearly indicated that p-aminobenzoic acid is considerably more active when it is added to urine *in vitro* than when it is excreted in urine following ingestion.

#### *Effect of ingestion of para-aminobenzoic acid on the pneumococcal activity of the blood of subjects receiving sulfonamides*

Bactericidal tests were carried out on defibrinated blood, as in previous studies (9). In some instances, subjects were selected whose blood was previously shown to lack pneumococcal power for the pneumococcus strains used. In other instances, blood samples, the pneumococcal properties of which were unknown, were inactivated before the test by heating at 56° C. for ½ hour, or by storing at icebox temperature for 72 hours so as to eliminate any bactericidal action that might be residing in the fresh blood. The essential details and the results of four separate experiments are shown in Table IV.



*Experiment I.* Subject E. C. was a normal adult whose freshly shed blood was shown on previous occasions to be completely devoid of pneumococcal action for the stock type III pneumococcus. He was given 5.0 grams of sodium sulfapyridine intravenously, followed in  $\frac{1}{2}$  hour by 2.0 grams of *p*-aminobenzoic acid orally. Blood samples were obtained immediately before each of the two drugs were given and also 1, 2 and 5 hours after the *p*-aminobenzoic acid was ingested. All the bloods were then tested for pneumococcal action on the stock type III pneumococcus.

The control blood again had no pneumococcal action. Following the intravenous injection of the sodium sulfapyridine, 0.5 ml. of this subject's blood inhibited the growth of 100,000 organisms for 48 hours and killed 10,000. The blood samples drawn 1 and 2 hours after the ingestion of the *p*-aminobenzoic acid showed complete loss of the killing power acquired as a result of the administration of sulfapyridine. The last blood, taken after 5 hours, however, again showed marked killing power. It was demonstrated in the previous section (*cf.* Table II) that *p*-aminobenzoic acid in this dose cannot be detected in the blood 5 hours after its oral administration. On the other hand, effective concentrations of sulfapyridine are still present in the blood for a much longer period following the intravenous injection of 5 grams of this compound (12).

*Experiment II.* Subject J. E. was another normal adult in whom a preliminary blood sample showed no killing power for type V pneumococcus. This subject was likewise given 5.0 grams of sodium sulfapyridine intravenously, followed this time by 1.0 gram of *p*-aminobenzoic acid by mouth. Blood samples were taken as before, except that the last blood was taken 4 hours after the *p*-aminobenzoic acid and each sample was heated at 56° C. for 30 minutes after it was drawn. These pneumococcal tests were done with the stock type V strain.

Again the control blood lacked pneumococcal action. In the first sample obtained after the intravenous administration of the 5.0 grams of sodium sulfapyridine, 0.5 ml. of this subject's blood killed 1000 pneumococci. The blood obtained 1 hour after the *p*-aminobenzoic acid ingestion lacked all bactericidal or even bacteriostatic action. The bloods taken after 2 and 4 hours were unable to kill more than 10 pneumococci per milliliter, but showed some bacteriostasis.

*Experiments III and IV.* These experiments were carried out with the blood of 2 patients who

were receiving sulfathiazole and were also the subjects of studies on the effect of *p*-aminobenzoic acid on toxic reactions. The stock type III pneumococcus was used. Initial bloods taken during the sulfathiazole therapy and before the administration of *p*-aminobenzoic acid showed moderate degrees of pneumococcal activity consistent with the concentrations of sulfathiazole which these blood samples contained. In both patients, bloods taken after the administration of *p*-aminobenzoic acid lost almost all of this pneumococcal action. In Subject S. L. (Experiment III) the second blood was obtained 1 hour after two 2-gram doses had been given, and in Subject L. C. the second blood was obtained 1 hour after five doses of 1.0 gram each had been given at 2-hour intervals (Experiment IV).

These experiments demonstrate that after the ingestion and absorption of *p*-aminobenzoic acid the bactericidal and bacteriostatic effect of sulfonamides on the pneumococcus is inhibited in the blood just as it is on addition of *p*-aminobenzoic acid in the test tube.

*Attempts to overcome and to prevent sulfonamide fever and rash by the administration of para-aminobenzoic acid*

Fildes (8), on the basis of the studies of Woods and Selbie referred to previously, made the suggestion that *p*-aminobenzoic acid might inhibit not only the bactericidal action of the sulfonamides but also their toxic effects in human tissues. The mechanism by which such toxic effects on body tissues are brought about is not known, and its relation to the mode of action of the drugs on bacteria is, therefore, not clear; nor is the same mechanism necessarily involved even in the different manifestations of toxicity. Drug fevers and rashes were selected for the present study because: (1) they are objective manifestations, (2) they subside fairly promptly on withdrawal of the offending drug and (3) they can usually be reproduced in the same patient, at least with the same drug. It is recognized, however, that these effects sometimes clear up while the drug is being continued.

Patients exhibiting either or both of these signs while under treatment with sulfapyridine or sulfathiazole were maintained on the same dose of

these drugs and given, in addition, 1 or 2 grams of *p*-aminobenzoic acid by mouth every 2 or 3 hours. The latter amounts were more than sufficient to overcome completely the antibacterial effects of the chemotherapeutic agents in the blood and urine *in vitro* (cf. Experiments III and IV in Table IV). Nevertheless, neither the fever nor the rash was influenced. When the sulfonamide was discontinued, the toxic signs cleared up in each instance, irrespective of whether the *p*-aminobenzoic acid doses were maintained or stopped.

Some of the patients, after a lapse of a week or longer without any drugs, were subsequently given regular large doses of *p*-aminobenzoic acid first, and later, in addition, were given some of the same sulfonamide drug which had previously produced fever and a rash. There were no untoward effects from the *p*-aminobenzoic acid while it was being taken alone, but the fever and rash regularly recurred within a few hours after the sulfonamide was given, even in spite of the continued administration of the *p*-aminobenzoic acid. Again, the toxic symptoms cleared when the sulfonamides were discontinued, regardless of whether or not the *p*-aminobenzoic acid doses were maintained.

A number of subjects were given *p*-aminobenzoic acid alone in repeated doses for 3 days without any ill effects.

#### DISCUSSION

The present observations which concern the inhibition of the action of sulfonamides on bacteria by *p*-aminobenzoic acid are consistent with Fildes' hypothesis (8), which considers substances of this type as "essential metabolites" that are displaced at some stage of bacterial growth by adequate concentrations of sulfonamides. West and Coburn's (4) observations likewise fit into this theory. On the other hand, our studies thus far fail to substantiate the idea that *p*-aminobenzoic acid plays the same rôle in animal cells and that the same mechanism would explain such toxic reactions as the fever and rash that result from therapy in human cases. The possibility that other toxic effects of sulfonamides may be affected by *p*-aminobenzoic acid or by similar chemicals is not excluded.

The results of the present studies suggest some useful laboratory applications for the activity of

*p*-aminobenzoic acid. Body fluids, such as blood, urine, and spinal fluids obtained from patients under treatment, frequently contain relatively high concentrations of sulfonamide drugs. When explants are made in nutrient broth or agar plates, enough sulfonamide is sometimes carried over to delay or prevent the growth of organisms that may be present. In such circumstances the addition of *p*-aminobenzoic acid to the nutrient medium will overcome this sulfonamide action and allow full growth of any organisms, provided they are still viable. This should be useful in determining the success of sulfonamide therapy.

Although the fate of *p*-aminobenzoic acid in the body is not clearly known, there can be little doubt that the compound is excreted, at least partly, in altered form. Modifications of the amino and carboxyl groups are most likely to occur. When *p*-aminobenzoic acid is injected into rabbits, about 25 per cent of it is recovered in the urine in the acetylated form (13). The present studies indicate that humans also acetylate this compound. Drug excreted in the urine, when determined colorimetrically by Bratton and Marshall's method, yielded higher "total" than "free" readings, that is, after acid hydrolysis more amino groups were available for diazotization. On the other hand, when solutions of the compound were added *in vitro* and determined by the same method, the results were the same before and after acid hydrolysis. Woods (6) found that alterations in the structure of *p*-aminobenzoic acid modified its antibacterial activity, so that acetoaminobenzoic acid was only half as active as the original compound. Likewise, alteration of the carboxyl group modified this activity. The conversion of the carboxyl group of *p*-aminobenzoic acid to its amide reduced the activity by one-third. Free carboxyl groups are "detoxified" in the body by conjugation. Quick (14) showed that many of the substituted benzoic acids are excreted as glucuronates. It therefore seems likely that the decreased activity of *p*-aminobenzoic acid after excretion in the urine, as compared with its activity when added to urine, can best be explained as the result of chemical alteration in the body, which probably does not affect the determination of "free" *p*-aminobenzoic acid.

It is of interest that in urine no amount of

*p*-aminobenzoic acid up to and including 500 mgm. per cent inhibited the bactericidal action of 500 mgm. per cent of sulfathiazole. It must be added, however, that 500 mgm. per cent of *p*-aminobenzoic acid appears, in itself, to prevent growth of *B. coli* in urine, even in the absence of sulfathiazole. Likewise, sulfathiazole in a concentration of 500 mgm. per cent may be toxic to bacteria by some action other than the postulated interference with vital metabolic enzymatic action, namely, by some direct lethal action. This latter supposition suggests a possible use for sulfonamide concentrations of this order in the treatment of empyema and other focal purulent lesions in which sulfonamide therapy has not yet yielded satisfactory results. The failure of therapy in such cases is ascribed partly to the inhibitory effect of the purulent exudate on sulfonamide action. Two points must be considered, however, in this connection: (1) The number of bacteria present in such lesions is generally considerably greater than in our experiments in urine; and (2) there is no evidence at present that the inhibitor present in pus is identical with *p*-aminobenzoic acid or acts in the same manner.

#### SUMMARY AND CONCLUSIONS

1. There is a roughly linear relationship between the concentrations of sulfonamides that have bacteriostatic action on pneumococci in blood broth and the minimum concentrations of *p*-aminobenzoic acid which inhibit that action. The inhibitory effect of *p*-aminobenzoic acid is most marked against sulfanilamide, less against sulfapyridine and least against sulfathiazole.

2. The action of sulfapyridine on pneumococci can be nullified at any stage by the addition of *p*-aminobenzoic acid. The resulting growth of pneumococci is then identical with that of a similar number of viable organisms freshly inoculated in the same medium without sulfapyridine.

3. Para-aminobenzoic acid is readily absorbed after oral administration. Maximum blood levels are reached in 1 to 2 hours after ingestion. Excretion is rapid and is practically completed in 12 hours. Some of the compound is present in conjugated form in the blood and urine. The drug is found in greater concentration in the plasma than in the red blood cells.

4. Within certain limits, *p*-aminobenzoic acid inhibits the bacteriostatic effect of sulfathiazole on the growth of *B. coli* in human urine. Urine obtained after ingestion of *p*-aminobenzoic acid is considerably less active in this respect than urine to which the same concentration of drug has been added *in vitro*.

5. The pneumococcal action of human blood resulting from the administration of sulfonamide drugs can be overcome by the ingestion of *p*-aminobenzoic acid.

6. Toxic effects of sulfathiazole therapy, as manifested by fever and rash, were neither prevented nor cured by the administration of *p*-aminobenzoic acid in amounts sufficient to nullify the bacteriostatic action of the drug in the blood.

The chemical determinations were carried out by Miss Elizabeth Shaler Smith.

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Dr. K. Dobriner, to whom we are also indebted, confirmed spectroscopically the presence in these urines of *p*-hydroxyphenylpyruvic acid (bands in region: 2900 to 3250  $\text{A}^\circ$ ) and either *p*-hydroxyphenyllactic or *p*-hydroxyphenylacetic acids. Differentiation of the latter two acids could not be made by spectroscopic means since their absorption bands are practically identical (bands in regions: 2780 to 2820  $\text{A}^\circ$  and 2860 to 2890  $\text{A}^\circ$ ). Appropriately treated urines never showed the absorption bands characteristic of benzoic, hippuric, phenylpyruvic or homogentisic acids.

#### *Quantitative assay of intermediary metabolites*

Following identification of the abnormal constituents in the urine as *p*-hydroxyphenylpyruvic and *p*-hydroxyphenyllactic acids, suitable methods were elaborated for their quantitative determination. Total hydroxyphenyl compounds (tyrosine, *p*-hydroxyphenyllactic and *p*-hydroxyphenylpyruvic acids), expressed as tyrosine equivalent, were determined by the Folin and Ciocalteu (7) method as modified by Medes (5). The urine, made weakly acid with acetic acid, was shaken with Lloyd's reagent (1.5 grams per 100 cc. urine) (8) to remove coloring matter and impurities capable of reacting with mercuric sulfate in subsequent procedures. An aliquot of the filtrate containing the equivalent of 0.15 to 0.50 mgm. tyrosine was treated with mercuric sulfate as outlined by Medes (5). Comparison of color was made either in a colorimeter against a standard containing 0.5 mgm. tyrosine or in an Evelyn photoelectric colorimeter which had been standardized with pure tyrosine solutions of known concentration.

The results could be expressed in terms of total tyrosine equivalent since equimols of *p*-hydroxyphenylpyruvic and *p*-hydroxyphenyllactic acids gave the same intensity of color as tyrosine. The rate of color development differed, however, in the three compounds. Whereas the maximum color developed by *p*-hydroxyphenylpyruvic acid was attained approximately 20 minutes after the addition of sodium nitrite, it appeared almost immediately with tyrosine and *p*-hydroxyphenyllactic acid and remained practically unchanged during the succeeding 20 minutes. Readings were therefore made exactly 20 minutes after addition of the sodium nitrite.<sup>4</sup>

Reduction of phosphomolybdic acid in acid solution as given by Medes (5) was used for the determination of *p*-hydroxyphenylpyruvic acid. Preliminary treatment of the urine with Lloyd's reagent was carried out as outlined above. In the early part of the work, a hydroquinone

standard was used for colorimetric comparison. Under the experimental conditions, 1 mgm. of hydroquinone was found to be equivalent to 0.3 mgm. *p*-hydroxyphenylpyruvic acid. In later work, readings were made with the Evelyn photoelectric colorimeter, using as a standard of reference a curve prepared with solutions of pure *p*-hydroxyphenylpyruvic acid of known concentration. The pure acid was prepared by the method of Herbst and Shemin (9). The method as given is non-specific but it was found that the urines contained practically no other substances capable of reducing phosphomolybdic acid in acid solution. Normal urine, as well as urine following removal of *p*-hydroxyphenylpyruvic acid by ether extraction, gave values equivalent to less than 5 mgm. *p*-hydroxyphenylpyruvic acid per 100 cc. Homogentisic acid, which gives this reaction, was excluded by repeated qualitative tests.

The difference between the total hydroxyphenyl compounds (expressed as tyrosine) determined by the Millon reaction (5, 7), and the keto acid determined by the reduction of phosphomolybdic acid (5), afforded a quantitative measure of *p*-hydroxyphenyllactic acid, since the urinary excretion of tyrosine *per se* remained a relatively constant and insignificant quantity<sup>5</sup> and since other phenolic derivatives were not present in the urine in significant amounts.

Total nitrogen was determined by the macro-Kjeldahl method; amino acid nitrogen by Folin's (2) colorimetric method; organic acids by the method of Van Slyke and Palmer (10) and creatine and creatinine by the Folin method (11). Free and total phenols were estimated by the method of Folin and Denis (12). This procedure was later abandoned when it was found that pure *p*-hydroxyphenylpyruvic acid gave irregular values far in excess of its theoretical phenol content. Lewis and his coworkers (4a) have reported similar inconsistencies for phenylpyruvic acid with this method (12).

#### RESULTS

##### *Relation of protein intake to excretion of hydroxyphenyl compounds*

A typical example of the defect in the metabolism of tyrosine and phenylalanine of premature infants is presented in Table I. This infant excreted minimal amounts of hydroxyphenyl compounds when he was receiving human milk with a protein intake of 2.3 grams per kgm. per day

<sup>4</sup> This procedure was followed except in instances when the output of total hydroxyphenyl compounds (expressed as tyrosine) was less than 50 mgm. per 24 hours, a level arbitrarily regarded as insignificant. Under these conditions, the required use of a volume of urine in excess of 0.5 cc. resulted in the development of turbidity on standing for 20 minutes. The time of readings in these instances was reduced to one minute after addition of the sodium nitrite.

<sup>5</sup> On a number of occasions, tyrosine was determined by the Folin and Ciocalteu (7) method following removal of *p*-hydroxyphenyllactic and *p*-hydroxyphenylpyruvic acids from the urine by extraction with ether. Routine resort to this procedure was unnecessary since the content of tyrosine, when determined, was always less than 25 mgm. per 100 cc. urine (or 50 mgm. per 24 hours) in the premature infants studied, whether the diet consisted of human or cow's milk.

TABLE I

*Effect of protein intake on urinary excretion of hydroxyphenyl compounds*

Age	Weight	Diet	Nitrogen intake	Urine									
				Total urine N	Creatinine	Creatine	Amino acid	Organic acids	Phenols		Total hydroxyphenyl compounds expressed as tyrosine	p-Hydroxyphenylpyruvic acid	p-Hydroxyphenyllactic acid and tyrosine*
days	kgm.		mgm. per kgm. per 24 hours	mgm. N per kgm. per 24 hours				cc. N/10 per kgm. per 24 hours	milligrams per 24 hours				
									Free	Conjugated			
10	1.95	Human milk	366	66	3.5	0.2	7	37	9	4	12	3	9
11	1.98	Cow's milk	764	103	4.3	0	9	46	12	6	17	4	13
12	1.99	Cow's milk	838	184	4.5	0.1	10	47	22	6	40	8	32
13	2.03	Cow's milk	823	247	4.8	0.1	10	57	32	4	62	12	50
14	2.06	Cow's milk	811	297	4.8	0.1	10	58	31	5	63	13	50
17	2.17	Cow's milk	852	384	4.8	0.5	13	81	195	11	626	136	490
18	2.23	Cow's milk	831	413	4.6	0.6	13	86	215	12	698	155	543
19	2.26	Cow's milk	820	476	4.9	1.2	15	103	244	9	833	196	637
20	2.27	Cow's milk	816	490	4.6	1.5	16	142	262	10	869	225	644
21	2.28	Cow's milk	812	440	4.4	1.2	18	118	236	0	772	205	567

\* Since tyrosine *per se* was a minimal and relatively constant quantity, blank values following ethereal extraction of the urine never exceeding 50 mgm. per day on the diets of cow's milk,\* the figures in this column represent predominantly *p*-hydroxyphenyllactic acid.

(nitrogen X 6.25). When the diet was changed to cow's milk containing more than 5 grams protein per kgm. per day, the excretion of these substances increased—gradually for the first 4 days following the change, and then abruptly during the next 3 days. In this infant, a maximum level (869 mgm. hydroxyphenyl compounds expressed as tyrosine) was reached on the 9th day after the change of diet. The level of excretion of *p*-hydroxyphenylpyruvic acid was 225 mgm. on this day, representing 26 per cent of the total hydroxyphenyl compounds. The remaining 644 mgm. represented *p*-hydroxyphenyllactic acid and minimal amounts of tyrosine.

The output of total hydroxyphenyl compounds in the 18 premature infants in whom the defect was studied varied from indeterminable amounts to 1385 mgm. per day and for *p*-hydroxyphenylpyruvic acid from traces to 444 mgm. per day. In all observations, the output of the hydroxy acid notably exceeded that of the keto acid, the latter comprising less than 40 per cent of the total hydroxyphenyl compounds (expressed as tyrosine) except in two instances (44 and 48 per cent), and averaging 29 per cent for the 18 infants.

The content of free phenols in the urine, 90 per cent of which were ether-soluble, varied directly with the concentration of hydroxyphenyl com-

pounds as determined by the Millon reaction (Table I). Conjugated phenols, on the other hand, were constant within the limits of error of the method. The organic acid content of the urine also paralleled the excretion of hydroxyphenyl compounds.

The amino acid output in the urine never increased significantly, seldom exceeding 20 mgm. amino acid nitrogen per kgm. per day and maintaining a practically constant level irrespective of the magnitude of excretion of aromatic organic acids. Similarly, fluctuations in the output of creatine and creatinine were unrelated to the level of excretion of these acids. Total urinary nitrogen varied with the nitrogen intake and was related to the excretion of hydroxyphenyl compounds insofar as the latter were excreted only when the nitrogen intake was relatively high.

Table II and Figure 1 illustrate even more graphically the dependence of the defect on the level of protein, and more especially on the aromatic amino acid intake. The daily intake and output are expressed in terms of body weight (mgm. per kgm.) in the table and in absolute values in the figure (mgm. per 24 hours). This premature infant was fed human and cow's milk of varying protein content and correspondingly varying intakes of phenylalanine and tyrosine (13,

14) in successive periods. A striking parallelism is noted between the intake of these aromatic amino acids (expressed as tyrosine) and the uri-

TABLE II

Relation of intake to excretion of hydroxyphenyl compounds

Age*	Period	Diet	Intake		Hydroxy-phenyl compounds in urine
			Nitrogen	Tyrosine plus phenylalanine	
days			mgm. per kgm. per 24 hours	mgm. tyrosine per kgm. per 24 hours†	mgm. tyrosine per kgm. per 24 hours
8	1	Cow's milk (high protein)	839	482	255
9			839	482	346
10			839	482	356
12	2	Cow's milk (low protein)	412	237	189
13			403	232	
14			398	229	
15			385	221	
16	3	Human milk (low protein)	385	221	87
17			438	160	7
19			427	156	
20			424	155	
21	4	Cow's milk (low protein)	458	263	6
23			437	251	
24			437	251	
25			428	246	
26			418	240	
27	5	Cow's milk (moderate protein)	418	240	8
28			606	349	25
29			597	344	
30			606	349	
31			606	349	
33	6	Evaporated skimmed human milk (high protein)	593	342	166
34			579	334	230
35			856	314	208
36			690	253	207
37			743	272	166
38	7	Cow's milk (high protein)	772	445	107
39			760	437	155
40			758	436	240
42	8	Cow's milk (high protein)	899	517	309
43			860	495	394
					358

\* The weight of this infant was 2.20 kgm. at the start and 3.26 kgm. at the end of observations.

† According to the data of Holt and Howland (13), human milk contains 0.75 per cent lactalbumin and 0.5 per cent casein and cow's milk contains 0.5 per cent lactalbumin and 3.0 per cent casein. The amino acid content was calculated from data given by Mitchell and Hamilton (14) for cow's milk protein—lactalbumin: 1.9 per cent tyrosine and 1.2 per cent phenylalanine; casein: 6.5 per cent tyrosine and 3.9 per cent phenylalanine. According to the figures given by Womack and Rose (15) and Bernstein *et al* (16), the tyrosine content of milk proteins is appreciably lower than the above. Use of the latter figures, however, would not have affected the qualitative interpretation of the results.

nary output of hydroxyphenyl compounds (in terms of tyrosine equivalent) in the different periods. Following changes in diet, the lag in excretion previously noted (Table I) is also evident in this observation (Periods 2, 5, 6 and 7) and it may afford a possible explanation for the absence of hydroxyphenyl compounds in the urine in Period 4 when the diet was changed from human (Period 3) to cow's milk of similar protein (2.7 grams per kgm.) but higher aromatic amino acid content. It should be further noted that in Period 6, when the diet consisted of evaporated skimmed human milk of higher protein (4.8 grams per kgm.) but of similar aromatic amino acid content, the excretion of intermediary metabolites persisted throughout the period although in progressively reduced amounts. This observation, not conclusive in itself because of the brevity of the period,<sup>6</sup> suggests that the defect is directly related to the level of the aromatic amino acid intake, whether the dietary protein be of human or bovine origin, a suggestion which is supported by the feeding observations with pure amino acids which follow.<sup>7</sup>

*Ingestion of phenylalanine and tyrosine.* Following prolonged fore-periods of constant diet of cow's milk, 8 premature infants were given either *L*-tyrosine, or *D,L*-phenylalanine in a single dose varying from 0.23 to 2.00 grams per kgm. The results are summarized in Table III.

The ingestion of either of these aromatic amino acids resulted in the prompt appearance of intermediary metabolites, when absent in the fore-periods, and in their augmented excretion, when previously present. The peak excretion was attained in some infants in the first 24 hours following ingestion; in other infants it was delayed for 48 hours or longer. Furthermore, the heightened level of excretion persisted in some infants throughout the period of study; in others it was transient, returning to the initial level within 2 to 3 days after ingestion. Finally, the maximal level

<sup>6</sup> This period was curtailed because of the development of diarrhea which promptly subsided when the diet was changed to cow's milk (Period 7).

<sup>7</sup> The possibility that human milk contained accessory factors capable of ameliorating the metabolic anomaly was excluded by experiments in vitamin C-deficient guinea pigs fed unboiled human milk (17), as well as by the results obtained in Period 6 of this observation.

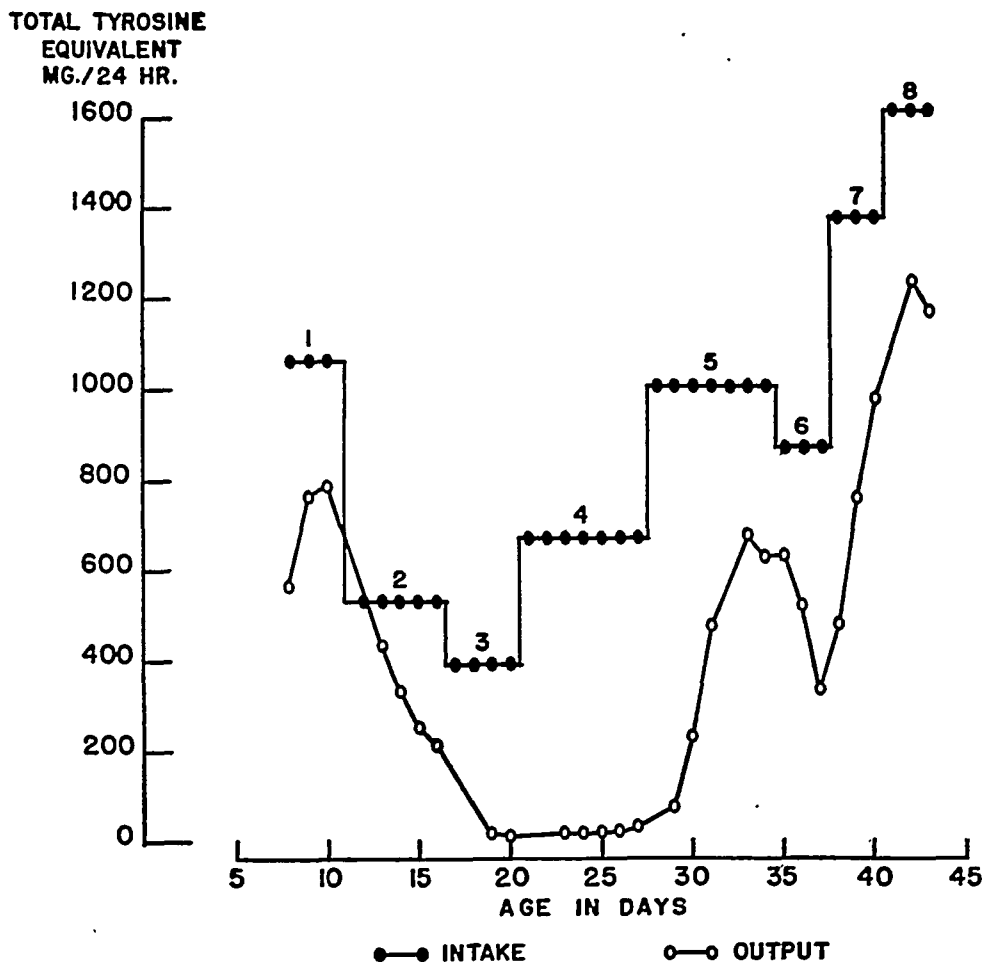


FIG. 1. EFFECT OF VARIATIONS IN THE INTAKE OF TYROSINE AND PHENYLALANINE (EXPRESSED AS TYROSINE) ON THE EXCRETION OF TOTAL HYDROXYPHENYL COMPOUNDS

of excretion was apparently not solely a function of the amount of amino acid administered. These differences in response of individual infants probably depended on the degree of vitamin C saturation of the tissues (18).

The fact that the urinary output of hydroxyphenyl compounds reached equally high levels in the feeding observations with phenylalanine as with tyrosine suggests that the normal growing human organism can oxidize the phenyl ring of phenylalanine to *p*-hydroxyphenyl derivatives, a conclusion already shown for animals by feeding (19) and liver perfusion (20) experiments.

It is interesting to mention that the administration of phenylalanine resulted in an increased output not only of hydroxyphenyl derivatives but also of phenylpyruvic acid, whereas the converse was

not demonstrable; *e.g.*, the administration of tyrosine augmented the output of phenolic derivatives without appreciably affecting the output of phenylpyruvic acid. Thus infant F. J. L. (Table III) excreted 104 mgm. phenylpyruvic acid (21) in 20 hours after the ingestion of 2.0 grams phenylalanine per kgm., but showed no appreciable excretion of this acid after the ingestion of tyrosine. This observation is in accord with expectation since phenylalanine, in contrast to tyrosine, is an essential amino acid (15) and the oxidation of phenylalanine to tyrosine is probably a biologically irreversible process (19).

The ingestion by these infants of other amino acids including glycine, methionine and the aromatic amino acid, tryptophane, in comparable or even larger dosage, failed to provoke the appear-



TABLE III

*Effect of ingestion of tyrosine and phenylalanine in premature infants*

Subject	Dose of amino acid		Hydroxy-phenyl compounds expressed as tyrosine	<i>p</i> -Hydroxy-phenyl-pyruvic acid	Subject	Dose of amino acid		Hydroxy-phenyl compounds expressed as tyrosine	<i>p</i> -Hydroxy-phenyl-pyruvic acid
	<i>total grams</i>	<i>grams per kgm.</i>	<i>mgm. per 24 hours in urine</i>			<i>total grams</i>	<i>grams per kgm.</i>	<i>mgm. per 24 hours in urine</i>	
<i>l</i> -TYROSINE					<i>d,l</i> -PHENYLALANINE				
F. Z.	1.00	0.45	30	12	F. J. L.	1.37	0.50	684	140
			218	112				688	151
			464	216				842	178
P. J. A.	2.50	1.00	973	348		5.78	2.00*	685	136
			1277	388				627	128
			1030	337				899	217
								1081	249
H. C. A.	2.60	1.00	905	203	W. B.	0.64	0.23	16	3
			1184	386				337	123
			1408	435				688	204
			1071	349				712	210
F. J. L.	1.19	0.50	498	98		2.21	0.82	831	256
			610	124				28	13
			695	116	R. S.			589	373
			592	118				839	396
G. C.	0.51	0.24	401	108					
			534	153					
			459	143					
			341	86					

\* On this day 104 mgm. phenylpyruvic acid were excreted in the urine.

ance or raise the urinary excretion of these intermediary products. The observation with tryptophane was of particular interest since no defect in its metabolism was demonstrated. The ingestion of 2.25 grams (1.0 gram per kgm.) of tryptophane by one infant (H. C. A.) was associated neither with an increased excretion of total hydroxyphenyl compounds or *p*-hydroxyphenylpyruvic acid nor with the appearance in the urine of indole, skatole (22) or kynurenic acid (23). In contrast with the lack of response to tryptophane, the ingestion of tyrosine in the same dosage by this infant a few days later (Table III) notably augmented the excretion of hydroxyphenyl compounds.

The excretion of tryptophane *per se* in the urine (7) amounted to 300 mgm., or 13 per cent of the ingested dose. The output of amino acid nitrogen, as determined, increased 43 mgm. in the 24 hours following ingestion, receding to the fore-period level during the succeeding 24 hours.<sup>8</sup>

<sup>8</sup> Although the output of amino acid nitrogen was never increased significantly in premature infants receiving

## COMMENT

Tyrosine and phenylalanine are among the few aromatic substances that ordinarily undergo complete oxidation in the human body. Aberrations in their metabolism are rare, the only well-known

cow's milk (20 mgm. or less per kgm. per day), or rarely after the ingestion of *l*-tyrosine, it did show an almost invariable tendency to rise following the ingestion not only of *l*-tryptophane but also of *d,l*-methionine and *d,l*-phenylalanine. In one observation with *d,l*-methionine, the increase in amino acid nitrogen excretion accounted for 27 per cent of the ingested dose of 1.0 gram per kgm. The increase in amino acid content of the urine in 6 of 7 observations with *d,l*-phenylalanine represented from 19 to 42 per cent of the ingested dose, the average of the 7 observations being 22.4 per cent. In 3 observations with glycine, the extra amino acid in the urine represented 4 to 12 per cent (average 7.5 per cent) of the ingested dose. A significant augmentation in excretion of amino acid nitrogen occurred, with one exception (*l*-tryptophane) following the ingestion of synthetic amino acids (*d,l*-methionine, and *d,l*-phenylalanine), suggesting that the unnatural enantiomorph present might have been a contributing factor (24).

instances being the congenital defects, alkaptonuria and phenylpyruvic oligophrenia (25). Even the artificial induction of defects entails considerable difficulty. Abderhalden (26) claimed to have isolated small amounts of homogentisic acid after the oral administration of 50 grams of tyrosine to a normal man but he was unable to produce alkaptonuria in rabbits and dogs. Papageorge and Lewis (27) also failed to produce alkaptonuria in rabbits but, when phenylalanine was fed to rats in amounts greater than 0.3 gram per 100 grams rat, alkaptonuria appeared within 2 to 29 days. Recent work by Sealock and Silberstein (28) has shown that alkaptonuria is readily produced in vitamin C-deficient guinea pigs by the ingestion of as little as 0.15 gram tyrosine per 100 grams animal per day. They obtained similar results in 2 normal human subjects on a vitamin C-deficient diet. The defect in the metabolism of tyrosine and phenylalanine exhibited by premature infants is not alkaptonuria since they never excreted homogentisic acid in significant amounts.

The path of the metabolism of tyrosine and phenylalanine is not definitely known but the available evidence indicates that *p*-hydroxyphenylpyruvic acid may be an obligatory intermediate (14). Kotake and his coworkers (3) found that rabbits excrete *p*-hydroxyphenylpyruvic acid in the urine after large doses of tyrosine and also *p*-hydroxyphenyllactic acid if *l*-tyrosine is ingested. When large amounts of phenylalanine were fed, phenylpyruvic acid was the principal intermediary product appearing in the urine but after several days of continuous administration of the amino acid, *p*-hydroxyphenylpyruvic acid was also excreted.

Lewis and his coworkers (4a) found phenylpyruvic acid in the urine of rabbits after feeding phenylalanine but the intermediary metabolite following tyrosine was not *p*-hydroxyphenylpyruvic acid but, presumably, *p*-hydroxyphenyllactic acid. Sealock and Silberstein (29) later demonstrated that vitamin C-deficient guinea pigs excrete *p*-hydroxyphenylpyruvic and *p*-hydroxyphenyllactic acids in addition to homogentisic acid after the administration of either tyrosine or phenylalanine.

A single instance of the spontaneous excretion

of *p*-hydroxyphenylpyruvic acid in a human was reported by Medes (5). She applied the term "tyrosinosis" to this anomaly which was characterized by the continuous excretion of about 1.6 grams *p*-hydroxyphenylpyruvic acid of endogenous origin, the excretion rising with ingestion of *p*-hydroxyphenylpyruvic acid, tyrosine or proteins. Only when the urinary output of *p*-hydroxyphenylpyruvic acid reached the high level of approximately 3 grams per day was tyrosine also excreted. *p*-Hydroxyphenyllactic acid was excreted still later after the subject had been on a high protein diet for several days or had received 10 grams of tyrosine per day for at least 2 days. Homogentisic acid was not excreted.

The defect in metabolism of tyrosine and phenylalanine observed in premature infants differs from the aforementioned defects, both spontaneous and artificially produced, in that more *p*-hydroxyphenyllactic acid than *p*-hydroxyphenylpyruvic acid was excreted and that significant amounts of homogentisic acid have not been found in their urine. It is, of course, conceivable that the failure of these infants to excrete the latter acid may have been due to the relatively low intake of its precursors. Papageorge and Lewis (27) found an intake of 0.3 gram phenylalanine per 100 grams rat per day necessary to produce it in rats. In Sealock's experiments (29) with scorbutic guinea pigs, 0.15 to 0.33 gram of tyrosine or phenylalanine per 100 grams of body weight provoked alkaptonuria. The diets fed to premature infants averaged 5 grams protein per kgm. per day. Using the previously quoted published data, this average intake may be calculated to contain the equivalent of about 0.5 gram of total tyrosine (tyrosine plus phenylalanine expressed as tyrosine) per kgm. per day. This level of ingestion is lower than the animal intake (1.5 to 3.3 grams per kgm.) and it is possible that with prolonged addition of extra tyrosine or phenylalanine to the diet, these infants might also have shown alkaptonuria. Single doses of as much as 2 grams per kgm. of either tyrosine or phenylalanine did not lead to the excretion of significant amounts of homogentisic acid.

The clinical significance of these findings in infants and the circumstances under which the de-

fect occurs and disappears will be discussed in the succeeding paper.

### SUMMARY

Premature infants receiving diets of relatively high protein content (5 grams or more per kgm. per day) exhibit a spontaneous defect in their metabolism of tyrosine and phenylalanine. The defect is manifested by the excretion of *l*-*p*-hydroxyphenyllactic and *p*-hydroxyphenylpyruvic acids in the urine. It may be accentuated by feeding these amino acids in pure form.

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# A DEFECT IN THE METABOLISM OF TYROSINE AND PHENYLALANINE IN PREMATURE INFANTS. II. SPONTANEOUS OCCURRENCE AND ERADICATION BY VITAMIN C<sup>1, 2</sup>

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In the preceding paper (1) an account was given of an aberration in the metabolism of tyrosine and phenylalanine in premature infants characterized by the urinary excretion of *l-p*-hydroxyphenyllactic and *p*-hydroxyphenylpyruvic acids. Data on the isolation, identification and properties of these intermediary metabolites and the chemical methods employed for their quantitative assay were presented. Earlier studies (2) had shown an irregular exhibition of the defect in premature infants fed high protein diets and this finding, together with observed variations in the magnitude and duration of excretion of abnormal metabolites in infants receiving equivalent protein intakes, pointed to the importance of other dietary factors besides the level of ingested protein. Later studies (3) demonstrated the curative properties of vitamin C.

This paper presents data which establish the following facts: (1) the prevalence and persistence of the defect in premature infants fed high protein diets (5 grams or more per kgm.) devoid of vitamin C; (2) its absence in full term infants receiving similar diets; (3) its production in the latter infants by feeding the pure amino acids, *l*-tyrosine or *d,l*-phenylalanine; (4) the specificity of vitamin C in preventing and eradicating the defect.

## METHODS

**Subjects.** Thirteen healthy male premature infants ranging in age from 6 to 31 days, and weighing from 1.49 to 2.22 kgm. at the start of observations, were studied on diets of vitamin C-free cow's milk throughout periods of from 4 to 69 days for a total of 378 days. The infants resided in a constant temperature and humidity room and were under the supervision of 4 spe-

cially trained nurses whose sole duties consisted of the preparation of the diets, collection of the urine and general care of the infants. Only one or occasionally 2 infants were observed at one time.

Twelve male full term infants ranging in age from 9 to 195 days, and weighing from 2.78 to 6.88 kgm. at the start of observations, were studied on similar diets in periods of from 1 to 28 days for a total of 83 days. The infants had completely recovered from the conditions for which they had been admitted to the hospital (respiratory infection, bronchopneumonia, hemorrhagic disease of the newborn, malnutrition, hypertrophic pyloric stenosis), and they were thriving during the periods of observation. Two of the full term infants who were studied on the basal diets of cow's milk were also studied following the ingestion of phenylalanine or tyrosine.

**Diets.** The basal diets consisted of dilutions of cow's milk supplemented with dextrimaltose or cane sugar. The total fluid and caloric intakes averaged 150 cc. and 120 calories per kgm. per 24 hours and were adequate to permit satisfactory weight gains. Protein supplied from 15 to 20 per cent,<sup>3</sup> butter fat or olive oil (olac) from 20 to 40 per cent, and carbohydrate the remaining 40 to 60 per cent of the calories. Twenty drops of a vitamin A and D concentrate (percomorph oil) were given daily. No vitamin C was added to the diets and analyses of the milk preparations showed the absence of ascorbic acid (4). Foreperiods of constant diet of at least 3, and usually many more days, preceded the onset of observations.

**Supplements.** The effect of vitamin C on the excretion of hydroxyphenyl compounds was determined in 10 premature and 2 full term infants, none of whom had previously received vitamin C in their diets. After the abnormal metabolites had appeared in the urine and were present in large amounts, vitamin C was given by mouth or parenterally in daily amounts ranging from 10 to 500 mgm. *l*-ascorbic acid to the point of eradication of these substances from the urine. Besides these studies

<sup>3</sup> Aliquot samples of the pooled milks were analyzed for nitrogen (Kjeldahl). The protein intake (nitrogen X 6.25) approximated 5 to 6 grams per kgm. per 24 hours and the calculated content of phenylalanine plus tyrosine (expressed as tyrosine) (1) averaged 500 mgm. per kgm. The following preparations of cow's milk were used: powdered whole milk, powdered half-skimmed milk (alacta), olac and half-skimmed olac.

<sup>1</sup> Presented in part at the meeting of the American Pediatric Society at Skytop, Pa., on May 3, 1940.

<sup>2</sup> Assistance in this work was given by the Children's Bureau, United States Department of Labor.

of the therapeutic action of vitamin C, its prophylactic effect was also noted in an additional 11 premature and 4 full term infants by the inclusion in their basal diets of supplements of vitamin C (10 or 20 mgm. *L*-ascorbic acid daily) for a varying number of days prior to observations.

In order to demonstrate the specificity of the curative properties of vitamin C, a number of infants who were excreting large amounts of hydroxyphenyl compounds during foreperiods of basal diets of cow's milk were given the following supplements, either singly or in combination and in high dosage: *D*-isoascorbic acid, thiamine, nicotinic acid, riboflavin, pyridoxine (vitamin B<sub>6</sub>), vitamin H (biotin), pantothenic acid, choline chloride, liver extract, yeast powder, rice polishings,  $\alpha$ -tocopherol and adrenal cortical extract (cortate).<sup>4</sup>

*Urine.* A detailed description of the method for collecting urine separately from feces in premature infants was given in previous papers (5). Urine was collected quantitatively from all of the infants throughout observations and analyzed at the end of each 24-hour period for the normal urinary constituents listed in the preceding paper (1), for total hydroxyphenyl compounds (tyrosine, *p*-hydroxyphenyllactic and *p*-hydroxyphenylpyruvic acids, expressed as tyrosine) (6, 7), and for the keto acid alone (*p*-hydroxyphenylpyruvic acid) (7) by the methods there outlined. The difference between the total tyrosine equivalent and the keto acid afforded a quantitative estimate of *p*-hydroxyphenyllactic acid (1).

*Blood.* Determinations of plasma ascorbic acid were made in a number of the infants<sup>5</sup> by the method of Mindlin and Butler (8). Roentgenograms of the long bones were taken at frequent intervals on 10 premature infants receiving the basal diets devoid of vitamin C.

## RESULTS

*Spontaneous excretion of hydroxyphenyl compounds by premature infants fed vitamin C-free cow's milk and the effect of vitamin C.* In Table I, the urinary excretion of total hydroxyphenyl compounds (expressed as tyrosine) and of *p*-hydroxyphenylpyruvic acid is presented for 13 premature infants receiving basal diets of vitamin C-free cow's milk, containing, with one exception (F. J. L.), from 700 to 900 mgm. of nitrogen per kgm. per day (4.4 to 5.6 grams of protein). All of the infants had been observed since birth and at no time prior to the onset of observations

had they received vitamin C either as supplements or in their previous diets of heated human or cow's milk (4). The periods of study on the basal diets extended from 2 to 61 days in individual infants, but to conserve space the table is arranged to show only the results obtained on the initial and final days of urinary collections. Following these basal periods, *L*-ascorbic acid was administered to 9 of the infants and the results are also shown in Table I.

The initial specimens of urine collected at ages ranging from 6 to 31 days contained total hydroxyphenyl derivatives equivalent to from 223 to 698 mgm. of tyrosine per day in all except 3 infants (H. A., F. J. L. and F. L.). The level of excretion in 2 of these infants was insignificant on the first day of observation (aged 19 and 22 days) but after 5 and 8 days, respectively, their excretion rate had attained notable proportions. In the remaining infant (F. L.) the output remained insignificant during a 5-day period of observation (10 to 14 days of age). It is possible that intermediary metabolites might have appeared had the observation been prolonged.

In all 12 infants who excreted hydroxyphenyl compounds, the excretion persisted at high levels for as long as observations were continued on the vitamin C-free diets of cow's milk. In one infant (J. S.) it extended to 78 days of age; on this day he excreted 929 mgm. of tyrosine. The range of output in the remaining 11 infants varied from 291 to 1327 mgm. in the final specimens of urine collected on the 12th to the 53rd day of life. The body weight of 6 of the 12 infants at the termination of these basal observations had reached levels well in excess of 2.5 kgm., the birth weight customarily used to differentiate full term from premature infants.

The output of *p*-hydroxyphenylpyruvic acid ranged from 36 to 175 mgm. per day in the initial specimens of urine in 10 of the infants and reached levels as high as 444 mgm. in the final specimens (J. S.). The excretion of keto acids for individual infants comprised from 15 to 34 per cent of the total hydroxyphenyl compounds, the remaining 66 to 85 per cent being almost entirely *p*-hydroxyphenyllactic acid (1).

*Effect of vitamin C.* The parenteral or oral administration to 9 infants of vitamin C in single

<sup>4</sup> The authors wish to thank Drs. R. R. Sealock, P. György, V. du Vigneaud, C. P. Rhoads, Mead Johnson Co., the Merck Chemical Co. and Eli Lilly and Co. for supplying generous amounts of one or more of these products.

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## METABOLISM OF TYROSINE AND PHENYLALANINE

TABLE I

*Spontaneous excretion of hydroxyphenyl derivatives by premature infants fed vitamin C-free milk. Effect of vitamin*

Sub- ject*	Age	Weight	Diet		Urine		Plasma ascor- bic acid	Sub- ject*	Age	Weight	Diet		Urine		Plasma ascor- bic acid
			Nitrogen intake	<i>L</i> -Ascor- bic acid†	Total hydroxy- phenyl com- pounds expressed as tyrosine	<i>p</i> -Hy- droxy- phenyl- pyruvic acid					Nitrogen intake	<i>L</i> -Ascor- bic acid†	Total hydroxy- phenyl com- pounds expressed as tyrosine	<i>p</i> -Hy- droxy- phenyl- pyruvic acid	
	days	kgm.	mgm. per kgm. per 24 hours	mgm.	mgm. per 24 hours		mgm. per 100 cc.		days	kgm.	mgm. per kgm. per 24 hours	mgm.	mgm. per 24 hours		mgm. per 100 cc.
P. J. A.	6	2.00	752		223	36		J. S.	17	2.08	738		434	89	0
	38	3.02	903		953	145			78	3.96	787		929	444	
	39-41			500					79-80			100			0
	40	3.18	857		67	33			81	4.00	779		68	25	
H. C. A.	41	3.23	844		33	15		J. C.	8	2.20	839		562	112	
	6	1.80	809		244	39			47	3.48	806		1327	377	
	46	3.09	827		1067	142	0		48-53			95			
	47-49			500					54	3.76	837		1258	160	0.3
M. P.	48	3.20	798		38	6		T. R.	54-55			50			
	49	3.24	788		32	6	0.3		56	3.78	832		294	120	0.3
	11	1.98	898		525	94			57	3.88	811		38	14	0.3
	12	1.99	893		627	80			21	2.16	918		698	175	
R. M. G.	13-14			150				W. B.	46	2.96	901		1159	351	
	15	2.05	868		30	3			47			500			
	16	2.10	845		24	Trace	0.1		48	3.00	889		907	152	
	18	1.78	925		319	85			49	3.01	886		920	152	0
R. A. G.	21	1.88	875		477	132	0	H. A.	24	3.04	878		1126	157	
	22-25			150					50	3.03	880		1120	143	
	26	2.10	784		36	5	0.1		51			500			
	38	2.54	814		714	114			52	3.04	878		937§	146§	
L. K.	38-39			400				F. J. L.	22	1.69	486		21	3	
	39	2.62	791		38	6			30	2.00	616		149	32	
	18	1.97	876		606	146	0		33	2.11	584		291	61	
	20	2.04	846		534	136			10	2.22	693		16	4	
L. K.	21			100				F. L.	14	2.49	742		32	6	0
	22	2.11	818		48	8									
	30	2.44	779		612	185	0								
	31	2.52	753		340	120	0.3								
L. K.	33			200				F. L.							
	34	2.64	719		40	8									
	31	1.82	932		437	103									
	53	2.70	801		744	101									
L. K.	54			275‡				F. L.							
	55	2.84	880		28	Trace	0.1								

\* Data on the first 5 subjects were presented in a previous communication (3).

† *L*-Ascorbic acid was given hypodermically in all infants except T. R. and J. C. in whom it was given orally.

‡ Two hundred mgm. *L*-ascorbic acid were given hypodermically; 75 mgm. orally.

§ Twelve-hour periods.

|| *d*-Isoascorbic acid.

¶ Vitamin C given at a later date caused the disappearance of hydroxyphenyl compounds.

or divided doses totaling 100 to 500 mgm. *L*-ascorbic acid resulted without exception in the prompt eradication of abnormal metabolites from the urine. In 2 infants (R. M. G. and R. A. G.) the cycle of appearance of urinary hydroxyphenyl derivatives with omission of vitamin C, and disappearance with its resumption, was demonstrated on two occasions. In another infant (J. C.), the oral administration of 95 mgm. of *L*-ascorbic acid in the course of 6 days was without demonstrable effect but continued administration of an additional 75 mgm. on the 3 succeeding days resulted

in the customary suppression of abnormal urinary metabolites.<sup>6</sup>

The oral administration of 500 mgm. of isomeric *d*-isoascorbic acid to one infant (T. R.) was accompanied by a transient decrease of approximately 20 per cent in the excretion of hy-

<sup>6</sup> The prophylactic action of vitamin C was also demonstrated in a group of 11 premature infants whose basal diets of heated cow's milk had been supplemented by a total of at least 70 mgm. *L*-ascorbic acid prior to observations. In 7, the spontaneous excretion of hydroxyphenyl compounds was entirely prevented by the prior administration of 70 to 100 mgm.



TABLE II  
Excretion of hydroxyphenyl derivatives by full term infants fed vitamin C-free cow's milk

Subject	Age	Weight	Nitrogen intake	Urine		Plasma ascorbic acid	Remarks
				Total hydroxyphenyl compounds expressed as tyrosine	p-Hydroxyphenylpyruvic acid		
	days	kgm.	mgm. per kgm. per 24 hours	mgm. per 24 hours		mgm. per 100 cc.	
W. Br.	9	3.59	819	15	Trace	0.1	Hemorrhagic disease of the newborn.
M. S.	13	4.94	865	39	19		Hemorrhagic disease of the newborn.
B. B.	16	3.70	869	12	13		Hemorrhagic disease of the newborn.
B. L.	21	3.16*	812	72	20	0.1	Birth injury.
G. T.	27	4.30	851	17	6		Cleft palate.
R. J.	30	2.78	1066	24	Trace		Feeding problem.*
J. R.	36	3.71	876	24	7		Staphylococcus sepsis; bronchopneumonia.
P. K.	42	3.86	868	14	4	0.1	Pyloric stenosis, postoperative.
B. C.	58	4.30	955	19	2		Conjunctivitis.
V. P.	143	6.88	820	24	5	0.1	Bronchopneumonia.
V. D.	195	5.38	826	48	9	0.1	Malnutrition; respiratory infection.
J. O.	36	3.42	900	1171	350	0	Feeding problem; convulsions.
	51	3.90	903	1450	449		
	53	500 mgm. l-ascorbic acid orally					
		4.00	881	45	7	0.3	

\* On this day the infant received l-ascorbic acid (20 mgm.) for the first time.

droxyphenyl compounds, with a return to the foreperiod level on the 3rd day following treatment (over 1100 mgm. in 24 hours). Five days later, an equal oral dose of l-ascorbic acid abolished the excretion of these substances within 48 hours. The minimal effect of d-isoascorbic acid on the metabolism of aromatic amino acids (9) is commensurate with its recognized lesser antiscorbutic potency (10).

Ascorbic acid was not detectable in the blood plasma of 6 infants in the vitamin C-deficient periods, and in 5, correction of the metabolic error by the vitamin was not accompanied by appreciable elevations of the plasma levels which remained at 0 to 0.1 mgm. per 100 cc. even after 100 mgm. or more of l-ascorbic acid had been given. The rise in the ascorbic acid content of the plasma of 3 other infants following administration of the vitamin did not exceed 0.3 mgm. per 100 cc. The complete absence or slight elevation of the ascorbic acid content of the blood plasma in the face of repair of the defect in cellular metabolism is in accord with published data (11) stating that replenishment of intracellular stores precedes and is not necessarily reflected in the plasma levels.

Roentgenograms of the long bones of 10 infants were, as might have been expected from their ages (12), essentially negative. It is well known, however, that a prolonged period may intervene between histological manifestations of scurvy and perceptible roentgenographic changes (13).

*Absence of spontaneous excretion of hydroxyphenyl compounds by full term infants.* In Table II are presented the results obtained in a group of 12 full term infants ranging from 9 to 195 days of age who were fed cow's milk mixtures devoid of vitamin C, and with a protein intake (5.1 to 6.6 grams per kgm. per day) comparable to that of the premature infants. None of these infants had previously received vitamin C in their diets or as supplements, with one exception (V. P.) who had been breast-fed until his admission to the hospital for bronchopneumonia 3 weeks prior to observation. In contrast to the results obtained in premature infants, 11 of the 12 subjects excreted insignificant amounts of hydroxyphenyl derivatives.<sup>7</sup>

<sup>7</sup> The single infant (J. O.) who excreted large amounts of aromatic metabolites was classified as a full term infant because his birth weight of 2530 grams exceeded

The ability of full term infants and the failure of premature infants to metabolize aromatic amino acids completely under similar conditions of diet cannot be fully explained at present, but two possibilities may be mentioned. One presupposes a more complete development with full fetal maturation of the enzyme systems involved in the breakdown of these amino acids analogous to the differential rate of fetal development of the chain of enzymes concerned with the metabolism of purines (14) and other substances. The persistent failure of hydroxyphenyl compounds to appear in significant amounts in the urine of one full term infant of 6½ months (V. D.) who, according to his history, had been on a vitamin C-deficient diet from birth and who presumably was in an extreme state of vitamin C desaturation (11b), and the prompt eradication of these substances from the urine of another full term infant (G. T., Table V) by the administration of a vitamin C-free liver extract, suggest that full fetal maturity may bring with it a factor or factors, not solely dependent on vitamin C, capable of completing the degradation of aromatic amino acids. The failure of vitamin C to suppress the urinary excretion of the aromatic amino acid metabolites, homogentisic acid and phenylpyruvic acid in the inborn anomalies, alkaptonuria (15) and phenylpyruvic oligophrenia (16), has already been established.

The second, and perhaps more likely possibility, presupposes quantitative differences in the body stores of vitamin C in premature and full term infants. This concept is supported both by analogy to the known differences in body storage of calcium and other substances in fetuses of different ages (17) and by direct evidence. Tissue analyses (18) have shown that the ascorbic acid content of the livers of premature infants at or shortly after birth is lower than that of full term infants under similar conditions of maternal diet. Tolerance tests indicate that young premature infants excrete only 10 to 15 per cent of a test dose

the standard of 2500 grams (5½ lbs.) statistically used to divide premature from full term infants. His history stated, however, that "he was born 2 to 3 weeks before term and required incubator care." The oral administration of 500 mgm. L-ascorbic acid to this infant on the 52nd day of life promptly abolished the urinary excretion of intermediary metabolites (Table II).

of vitamin C in 24 hours (19) in contrast to 28 per cent excreted by full term infants following an equivalent dosage (20). The absence of hydroxyphenyl derivatives in the initial urine specimens of some premature infants (Table I), and their subsequent appearance with no change in diet, may be explained on the basis of progressive depletion of the vitamin C stores of the body. Furthermore, the excretion of these metabolites persisted in all of the premature infants for as long as they were observed; in one infant up to 78 days of age and 4.0 kgm. in weight. If one makes the reasonable assumption that, at this late age the enzyme systems of this premature infant had attained a stage of development commensurate with that of an infant born at term, the persistence of the defect indicates the fundamental position of vitamin C in completing the oxidation of aromatic amino acids in these infants.

*Effect of ingestion of tyrosine and phenylalanine by full term infants.* Since the ingestion of single doses of phenylalanine and tyrosine always increased the excretion of hydroxyphenyl compounds in premature infants on vitamin C-free diets of cow's milk (1), the effect of these amino acids was determined in 2 of the full term infants previously studied (Table II) under similar dietary conditions.

Both infants responded to ingestion of the amino acids (1.0 gram per kgm.) by a prompt and persistent rise in excretion of hydroxyphenyl compounds. Maximum levels of 1050 and 1750 mgm. were attained on the 7th and 12th days following ingestion, the maximal levels for *p*-hydroxyphenylpyruvic acid being 460 and 528 mgm. on the 4th and 6th days, respectively. In infant J. R., these substances were still present in large amounts (1343 and 353 mgm.) 18 days after the feeding. The ingestion by these 2 infants of a single dose of 1.0 gram of tyrosine or phenylalanine per kgm. was apparently sufficient to disrupt the enzymatic mechanisms responsible for complete oxidation of aromatic amino acids and to initiate a persistent change in their cellular metabolism, whereas the daily ingestion of slowly absorbed, divided doses of one-half this amount (0.5 gram per kgm. as components of the protein of the basal diet of cow's milk (1)) did not flood the cells to the point of precipitating the defect in

TABLE IV—Continued

Age	Medication†									Total hydroxyphenyl compounds expressed as tyrosine	p-Hydroxyphenylpyruvic acid
	Thia- mine	Ribo- flavin	Nico- tinic acid	B <sub>6</sub>	Choline Cl	II	Panto- thenic acid	Crude liver extract	Crude liver extract plus thiamine and riboflavin		
days	mgm.	mgm.	mgm.	mgm.	mgm. per kgm.	cc.	mgm.	cc.	cc.	mgm. per 24 hours	mgm. per 24 hours
43	5.0	2.0	10	10	0.5					880	175
44										868	169
47										842	188
48										875	189
49										822	204
50							10			850	241
51							10			748	197
52							10				
53							10				
54							10			773	222
55	5.0	2.0	10	10	0.5		10			789	255
56	5.0	2.0	10	10	0.5		10			770	248
57	5.0	2.0	10	10	0.5		10			816	288
58	5.0	2.0	10	10		4.0	10			774	262
62						4.0	10			666	228
63										1132	322
64								2			
65								2		654	165
66								2		932	233
67								1		930	236
68								1		866	226
69										1040	284
70										992	273
71										962	311
72									2		
73									2	1206	398
74									2	864	310
75									1	1022	380
76											
77										909	337
78										929	444
L-Ascorbic acid—100 mgm. by hypodermic											
81										68	25
83										51	23

In 2 of the premature infants, J. S. (Table IV) and L. K., treatment with 7 and 8 cc. of the liver extract (1 or 2 cc. daily) was entirely without effect on the excretion of aromatic metabolites; in another infant (W. B.) treatment with the same extract (2 cc. daily for 3 days) produced a moderate decrease in the daily output of these substances from 368 to 270 mgm., a decline of approximately 26 per cent.<sup>11</sup> The fourth premature

<sup>11</sup> On the day following the last injection of liver this infant received 1 gram *d*-isoascorbic acid orally. The abnormal metabolites completely disappeared from his urine and their excretion remained negligible for as long as 13 days thereafter when the observation was discontinued. Further studies on additional premature infants are being undertaken to determine whether these two agents are capable of exerting a synergistic effect

infant (T. R.) responded to the parenteral administration of the extract (2 cc. daily for 3 days) with a transient but definite drop of approximately 40 per cent from the daily foreperiod level of 764 mgm. to 456 mgm.

In contrast to the absent or moderate and transient responses of premature infants to injections of liver extract, one full term infant (G. T.), in whom the defect had been provoked by tyrosine ingestion, responded to comparable treatment (a total of 6 cc. in 4 days) with a prompt and persistent eradication of excretion of hydroxyphenyl compounds. Further observations

on the metabolic defect in these subjects, since the action of 0.5 gram *d*-isoascorbic acid alone (Table I) was only transient and slight.

TABLE V  
Effect of the administration of liver extract on the excretion of hydroxyphenyl derivatives

Subject	Age	Liver extract*	Urine		Subject	Age	Liver extract*	Urine	
			Total hydroxyphenyl compounds expressed as tyrosine	p-hydroxyphenyl- pyruvic acid				Total hydroxyphenyl compounds expressed as tyrosine	p-hydroxyphenyl- pyruvic acid
days	cc.	mgm. per 24 hours		days	cc.	mgm. per 24 hours			
PREMATURE INFANTS				PREMATURE INFANT					
L. K.	46		866	166	T. R.	32		764	246
	47		869	203		33		762	264
	48	2				34	2	694	207
	49	2				35	2	578	157
	50	1	765	154		36		456	127
	51	2	837	159		37		456	146
	52	1				38		473	162
	53		744	101		39		614	200
W. B.	22		398	131	FULL TERM INFANT				
	23		409	129	G. T.†	35		1050	290
	24		368	132		36	2	974	260
	25	2				37	2	646	173
	26	2				38	1	358	90
	27	2	367	120		39	1	50	9
	28		270	77		40		26	5
						41		25	5
				48			41	12	

\* Crude liver extract fortified with thiamine and riboflavin, given intramuscularly.

† Metabolic defect produced by ingestion of tyrosine on 29th day of life.

are at present being made on other full term infants. If the potency of liver extract<sup>12</sup> in the latter is substantiated, it will illustrate another point of difference between premature and full term infants.

#### COMMENT

An aberration in the metabolism of aromatic amino acids has been demonstrated in premature infants who are fed relatively high protein diets (5 grams or more per kgm. per day) in the form of cow's milk without supplements of vitamin C. This aberration is, as a rule, not spontaneously demonstrable in full term infants on similar diets but it may be artificially provoked in them by

<sup>12</sup> The liver extract which was used in these observations was tested and found to contain no vitamin C. Although reducing substances were present, the rate of color development differed from that of the vitamin. Furthermore, the manufacturer reported that the method of processing the liver extract undoubtedly destroyed any vitamin C originally present.

the feeding of *d,l*-phenylalanine or *l*-tyrosine. It consists in the inability of these subjects to oxidize these aromatic amino acids beyond the organic acid stage and is manifested by the appearance in their urine of the intermediary products, *p*-hydroxyphenylpyruvic and *l-p*-hydroxyphenyllactic acids. The absence of other intermediary metabolites (except phenylpyruvic acid following the ingestion of phenylalanine) has been established by appropriate tests. This metabolic defect may be prevented or, when present, may be promptly abolished by the administration of vitamin C in adequate dosage. Its reversibility differentiates it from the hereditary anomalies, alkaptonuria and phenylpyruvic oligophrenia, both of which have been shown not to be remediable by the administration of vitamin C (17, 18). The spontaneous occurrence of the defect in the premature infant provides a good medium for the study of the intermediary metabolism of aromatic amino acids in the growing human organism.

The reported data indicate that the metabolic error observed in infants is dependent on at least three factors: (1) the level of protein (aromatic amino acid) intake, (2) the degree of vitamin C saturation of the tissues, and (3) the maturity of the infant. The last factor may be solely a function of the second or it may be related in part to a more complete development in the full term infant of the enzyme systems involved in aromatic amino acid metabolism. The evidence favoring these alternate concepts has already been considered.

The reported observations do establish beyond any doubt the key position of vitamin C in the metabolism of aromatic amino acids in the growing human organism. If the metabolism of these amino acids increases the vitamin C requirement (9), their higher content in cow's milk may contribute to the observed lower plasma levels of vitamin C in infants on this feeding than in those fed human milk (22) and to the increased incidence of scurvy in infants fed cow's milk. This possibility is at present being investigated. If substantiated, it will supplement the explanation usually offered for the above differences in the plasma ascorbic acid of infants fed human and cow's milk, namely, the difference in content of vitamin C of the two diets.

Demonstration of the fundamental importance of vitamin C in aromatic amino acid metabolism suggests the possibility of employing the exhibition of the metabolic defect, either spontaneous or induced, for the early detection of vitamin C deficiency in premature and young infants. Although the defect appeared in several infants as early as the first week of life, the fact that a relatively high intake of aromatic amino acids was required for exhibition of the defect sharply limits its usefulness as a test for vitamin C deficiency. The evidence does indicate, on the other hand, that exhibition of the defect represents an extreme degree of vitamin C desaturation of the tissues and that disappearance of abnormal urinary metabolites, when previously present, may constitute an early and sensitive sign of repair of intracellular vitamin C deficiency. The prompt correction of the metabolic error following vitamin C administration, in the absence of any appreciable rise in the ascorbic acid level of the plasma, indicates that the former effect is a much more delicate indicator of such repair than changes in blood plasma ascorbic acid levels.

The observations reported in this paper give rise to a number of intriguing but as yet highly conjectural questions. What is the relation, if any, between the demonstrated defect in the metabolism of tyrosine and phenylalanine and the morphologic changes characteristic of vitamin C deficiency, namely, improper formation of intercellular substance and collagen (23)? Since tyrosine is a precursor of both thyroxine and adrenalin, may the presence of the defect in premature infants contribute, under certain conditions, to their instability of body temperature regulation? Is the delicate transparency of the skin of premature infants related to improper utilization of melanin, another product of tyrosine? Does the observed increased excretion of aromatic organic acids in premature infants fed cow's milk play a rôle in their known tendency to develop severe rickets? If so, this observation may explain, in part at least, the frequently postulated importance of vitamin C in the development of rickets. Many more questions arise but they are all matters of surmise at present and they must await further study before meriting consideration.

## SUMMARY

*Premature infants.* Premature infants fed diets of vitamin C-free cow's milk containing 5 grams or more of protein per kgm. exhibited a spontaneous defect in metabolism of aromatic amino acids manifested by the excretion in their urine of 1-*p*-hydroxyphenyllactic and *p*-hydroxyphenylpyruvic acids. This defect was noted as early as the 6th day of life and persisted for as long as vitamin C was withheld. The administration of 1-ascorbic acid completely eradicated the defect without necessarily raising the plasma ascorbic acid levels. The administration of *d*-isoascorbic acid had a transient and partial effect, and the administration in large dosage, either singly or in combination, of thiamine, riboflavin, nicotinic acid, vitamin B<sub>6</sub>, vitamin H, pantothenic acid, choline chloride,  $\alpha$ -tocopherol, adrenal cortical extract, rice polishings, yeast powder and liver extract (orally) was without effect. In one of 4 infants, a crude liver extract by injection resulted in a transient but definite decrease in excretion of hydroxyphenyl compounds.

*Full term infants.* Full term infants fed similar diets showed no spontaneous defect in their metabolism of aromatic amino acids. The defect was precipitated by the ingestion of a single dose of 1.0 gram per kgm. of phenylalanine in one infant and of tyrosine in another. The artificially induced defect in these subjects was readily abolished by the administration of 1-ascorbic acid and, in one infant, by the parenteral administration of whole liver extract.

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# HYDROGEN ION CONCENTRATION OF THE DUODENAL CONTENTS UNDER FASTING CONDITIONS IN NORMAL PERSONS AND IN PATIENTS WITH DUODENAL ULCER: A COMPARATIVE STUDY<sup>1</sup>

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The experimental surgeon, working on animals, has contributed materially to our fund of information concerning the formation of peptic ulcer. He has shown that the withdrawal of alkaline duodenal secretion from the particular portion of the intestinal tract into which the stomach empties, and exposure of the intestinal mucosa to acid gastric chyme for abnormally long periods, lead to the formation of ulcer in a high percentage of experiments regardless of the surgical procedure employed (1 to 7). It has been concluded from these experiments that the hydrochloric acid poured from the stomach into the duodenum traumatizes the duodenal mucosa by chemical and mechanical action in such a way as to produce the ulcer, even though the existence of the conditions experimentally induced in animals has not been demonstrated in patients with duodenal ulcer.

In reality, the pathologic physiology underlying formation of duodenal ulcer has not been studied to any great extent in man. Only one fact has been clearly demonstrated, namely, that the average gastric acidity of patients with duodenal ulcers is greater than that of normal persons (8, 9). It has not been shown that the entrance of more than average amounts of acid from the stomach into the duodenum in patients with duodenal ulcer creates an abnormally acid condition in the duodenum.

Some effort has been made to determine the hydrogen ion concentration of the duodenal contents of normal persons but the information gained has been fragmentary (10 to 16). Determination of hydrogen ion values usually has been on single specimens obtained by duodenal intubation at long intervals or on block samples

collected over varying periods. In none of these observations has the behavior of values for pH been studied in relation to gastric acidity. Even less is known about the hydrogen ion concentration of the duodenal contents of patients with duodenal ulcer. Morton's work is the first and only attempt to study this problem so far as we can find (17). He reported that free acid was present in the duodenal contents of patients with peptic ulcer, whereas free hydrochloric acid was not recovered from the duodenal contents of persons without ulcer. His determinations were made one hour after an Ewald test meal and on single specimens obtained with the duodenal tube. These data obviously give an incomplete picture of the changes in hydrogen ion concentration of normal persons and of patients with duodenal ulcer.

In this investigation we propose to study the hydrogen ion concentration of the duodenal contents of normal persons with varying degrees of gastric acidity in order to obtain a norm, and of patients with duodenal ulcer in the hope of finding some variation from the normal behavior of the hydrogen ion concentration of the duodenal contents which might have some bearing on the causation of ulcer.

## MATERIAL

A total of thirty-four successful series of observations was performed on seven normal subjects, and twenty successful series of observations on thirteen patients with duodenal ulcer. The duration of the observations on normal subjects totaled eighty-seven hours and on patients with duodenal ulcer fifty-one hours.

## METHODS

The duodenal tube was introduced into the duodenum after a fast of twelve hours. That the tip of the tube was located in the second portion of the duodenum was confirmed by roentgenologic examination. The duodenal

<sup>1</sup> Abridgment of thesis submitted by Dr. Kearney to the Faculty of The Graduate School of The University of Minnesota in partial fulfillment of the requirements for the degree of M.S. in Surgery.



contents were withdrawn continuously, a negative pressure of 14 inches (36 cm.) of water being used. A flow of duodenal contents sufficient for determination of the values for hydrogen ion concentration was obtained at all times with the exception of a few short intervals during a total of more than 130 hours of continuous aspiration. In the early observations the pH value was determined on block samples at five- to ten-minute intervals by means of a platinum quinhydrone electrode with a Leeds-Northrup potentiometer. In a majority of the determinations, however, a Cameron glass bulb electrode was used; in these observations the contents from the duodenum were drawn through a small chamber into which the glass bulb electrode projected and at two-minute intervals the flow was interrupted for a few seconds sufficient to make the determination.

#### *Possible errors in data arising from technic*

The constant removal of duodenal contents shortens the time during which the hydrochloric acid normally remains in the duodenal cavity. This may diminish the response of the hormonal mechanism that stimulates the flow of bile and pancreatic juice or that which depresses the secretion of acid by the stomach. The pH values obtained may not, therefore, be the pH values in the undisturbed fasting duodenal contents. However, any error arising from the technic will affect in a similar manner values obtained from normal persons and from patients with duodenal ulcer and should not affect the validity of data for comparison of the behavior of values in the two groups of patients.

#### *Data from normal persons*

The gastric contents of normal Subject 1 did not contain free hydrochloric acid after stimulation either with the water carbohydrate meal or with histamine (Table I). During two series of observations lasting a total of nearly three hours, pH values varied from 6.7 to 7.7.

The gastric contents of normal Subject 2 obtained under fasting conditions or after stimulation with the water carbohydrate meal did not contain free hydrochloric acid, but after stimulation with histamine in doses of 0.1 mgm. per 10 kgm. of body weight the free hydrochloric acid attained a value of 26 clinical units and the 129 cc. of gastric contents recovered during the sixty minutes following stimulation contained 25 cc. of tenth normal hydrochloric acid (Table I). In spite of the fact that the stomach of this subject was able to secrete acid in small quantities after stimulation with histamine, the pH values fluctuated from 6.0 to 7.5 and did not fall below 6 during the entire period of the observations (three hours), thus remaining in practically the same range as did the values obtained from normal Subject 1 with true anacidity.

The gastric secretory activities in normal Sub-

TABLE I

*The gastric secretory activity and the percentage of total duration of observations during which various pH values of duodenal contents were obtained in seven normal subjects*

Subject	Modified Ewald meal*		Stimulation with histamine†			Number of series of observations	Total duration in minutes	Percentages of total duration of observations during which various pH values of duodenal contents were obtained								
	Total acidity	Free acidity	Volume	Total cc. tenth normal HCl	Highest concentration free HCl			1.1 to 2	2.1 to 3	3.1 to 4	4 and below	4.1 to 5	5.1 to 6	6 and below	6.1 to 7	7.1 to 7.9
1	6	0	cc.	0	0	2	168	0	0	0	0	0	0	0	29.7	70.3
2	6	0	129	25	26	1	182	0	0	0	0	0	0	0	85.8	14.2
3	54	48	86	99	112	9	1568	0	0	2.7	2.7	6.6	48.4	57.7	38.0	4.3
4	50	38	140	96	94	3	383	0	4.2	7.8	12.0	7.3	32.9	52.2	37.4	10.4
5	68	56	164	95	70	9	1448	5.2	7.6	8.3	21.1	11.6	45.2	77.9	22.0	0.1
6	70	50	160	123	86	4	709	0	7.8	14.7	22.5	17.1	19.4	59.0	32.5	8.5
7	96	80	195	196	120	6	751	7.5	11.3	14.0	32.8	13.3	15.2	61.3	34.0	4.7
Average 3, 4, 5, 6 and 7						34†	5209†	2.54	6.18	9.50	18.22	11.18	32.22	61.62	32.78	5.60

\* Values in clinical units one hour after ingestion of meal.

† Values obtained in the one-hour period after stimulation.

‡ Totals for all seven subjects.

jects 3, 4, 5 and 6 were similar to one another and moderate (Table I). The behavior of the pH values of the duodenal contents in these subjects is illustrated in Figure 1. The roentgenograms showed the tip of the tube to be in the second portion of the duodenum; the glass bulb electrode was used. During the first eight minutes a slightly cloudy amber fluid with pH values varying between 6.7 and 4.5 was obtained. The contents then changed in gross appearance, becoming very cloudy and containing much gastric secretion. The pH values dropped below 3 within ten minutes; then the gross appearance of the duodenal contents again changed, becoming amber, and the pH values rose to 5.5, a level at which they remained for the next sixty minutes. The material then grossly appeared to be a mixture of bile and gastric contents and the pH values dropped to about 3 or below for ten minutes; then a flow of clear amber fluid occurred and the pH values rose to 6.5 and remained at about this level until the end of the observation was reached.

The flow of acid gastric material from the duodenal tube was followed promptly by a flow of amber colored fluid in this and other observations on these subjects. This means that the entrance of acid gastric material into the duodenum promptly evoked a secretion of bile and pancreatic juice. The frequency and duration of the periods during which acid gastric material was obtained from the duodenum varied considerably. However, the excursions of pH values below 4 never lasted more than twenty minutes and 78 per cent of the excursions lasted ten minutes or less. The

tendency for the values to be maintained in the upper less acid levels is definite. The percentage of duration of each observation on each subject during which the pH values were below 4 was greater than 25 in only one observation (reaching 37 per cent). Moreover, the values remained above 6, 42.3, 47.8, 22.1 and 41 per cent of the time and fell below 4 only 2.7, 12.0, 21.1 and 22.5 per cent of the total time during which values were determined, respectively, in Cases 3, 4, 5 and 6 (Table I). In this connection it is interesting that the pH values fell below 2 in Case 5 only and then only for 5.2 per cent of the total time during which values were determined in this case.

It appears that the stomachs of these normal persons (Cases 3, 4, 5 and 6) were emptying acid into the duodenum only a little more than half the time. If free hydrochloric acid was entering the duodenum a greater part of the time than the figures in Table I indicate, the duodenal contents were present in such quantities and of such alkalinity that the quantity of free acid entering the duodenum could depress the pH values below 6 only a little more than half of the time. It also appears that the response of outflow of bile and pancreatic juice to the entrance of highly acid gastric contents into the duodenum was prompt and in quantities sufficient to prevent the existence of free hydrochloric acid (pH value below 4) in the lumen of the duodenum for long periods. Such behavior may be considered normal for healthy persons whose stomachs are capable of secreting the more moderate amounts of acid.

The free hydrochloric acid was obtained from

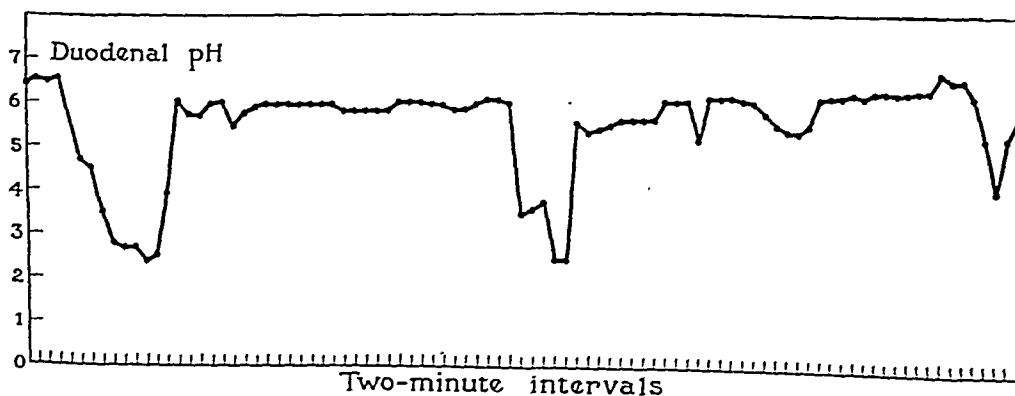


FIG. 1. THE TYPICAL BEHAVIOR OF VALUES FOR THE HYDROGEN ION CONCENTRATION OF THE DUODENAL CONTENTS OF NORMAL PERSONS WITH AVERAGE GASTRIC SECRETORY ACTIVITY

the stomach of normal Subject 7 in quantities well above the average for normal persons (Table I). Seventy per cent of the excursions of the pH values of the duodenal contents below 4 lasted ten minutes or less, a figure that is not very different from that (78) obtained in Cases 3, 4, 5 and 6. However, the excursions of pH values below 4 lasted longer than twenty minutes in three instances (twenty-five, thirty and thirty-two minutes). The percentage of duration of each observation during which the pH values were below 4 varied more widely in Subject 7 than in Subjects 3, 4, 5 and 6, the percentage being greater than 25 in three of six observations, whereas it was above 25 in only one of twenty-four observations in Subjects 3, 4, 5 and 6. The percentages of duration of observations 3 and 6, Subject 7, during which the pH values were below 4, were 50 and 51, respectively. Such percentages are seen in some subjects with duodenal ulcer but are far below those seen in most cases with this disease. The pH values were below 4 in a slightly greater percentage of the duration of all observations on Subject 7 (32.8 per cent) than in Cases 3, 4, 5 and 6 (2.7 to 22.5 per cent, Table I).

In other words, a greater secretion of acid by the stomach of normal Subject 7 than by the stomachs of normal Subjects 3, 4, 5 and 6 caused the duodenal contents of Subject 7 to be slightly more acid than those from normal Subjects 3, 4, 5 and 6. In spite of these differences, the pH values remained above 6 about the same percentage of time in Subject 7, as noted in the observations of Subjects 3, 4, 5 and 6, and the behavior of the pH values seen in Subject 7 was not far different from that encountered in normal Subjects 3, 4, 5 and 6. For the most part, the neutralizing and diluting mechanism in the duodenum of Subject 7 was almost as competent to take care of the larger amounts of acid pouring into the duodenum as was that of Subjects 3, 4, 5 and 6 to take care of the smaller amounts of acid entering their duodenums.

A constant, direct relationship cannot be demonstrated in these seven normal subjects between the gastric secretory activity and the behavior for pH values of the duodenal contents. It is obvious, however, that the behavior of the pH values of the duodenal contents is distinctly different in the case with low gastric secretory activity (Case

2) from the behavior of the pH values of the duodenal contents in the cases with about average gastric secretory activity (Cases 3, 4, 5 and 6). Moreover, the behavior of pH values of the duodenal contents is at least slightly different in the cases with average gastric acidity from that in the case with high gastric acidity (Case 7). The amount of acid gastric juice entering the duodenum seemingly does influence the behavior of the pH values in normal subjects.

#### *Data from patients with duodenal ulcer*

The behavior of the pH of the duodenal contents in Subject 8 with duodenal ulcer (Table II) resembles the behavior seen in the normal person with an acidity (Case 1, Table I) or that seen in the normal person with very low gastric acidity (Case 2, Table I). The gastric contents of Subject 8 contained 44 units of free acid after the modified Ewald meal but did not contain any free acid in the fasting state. This may have some bearing on the occurrence of the high pH values (above 6.5) in the three hours during which determinations were carried out and on the apparent failure of acid to enter the duodenum during this period.

The behavior of the pH values in Subject 9 with duodenal ulcer is similar to that seen in the normal individual with average gastric secretory activity in spite of the ability of the stomach of this patient to secrete acid in amounts greater than average. The diluting and neutralizing mechanism of the duodenum appears capable of caring for any excess secretion of acid that may have occurred in this series of observations.

In the first series of observations on Subject 10 with duodenal ulcer, the pH values were below 2, 81 per cent of the time, whereas in the second series of observations on the same subject the values were above 6, 100 per cent of the time. Such marked variability in the pH values was unusual; in other cases in which two series of observations were made the distributions of pH values were similar.

The behavior of the pH values in subjects with duodenal ulcer (Subjects 11 to 20, inclusive) was remarkably consistent and similar. The behavior is illustrated in the observations on Subject 16 with duodenal ulcer (Figure 2). The duodenal contents at the start obviously contained much

TABLE II

*The gastric secretory activity and the percentage of total duration of observations during which various pH values of duodenal contents were obtained in thirteen patients with duodenal ulcer*

Subject	Modified Ewald meal*		Stimulation with histamin†			Number of series of observations	Total duration in minutes	Percentages of total duration of observations during which various pH values of duodenal contents were obtained												
	Total acidity	Free acidity	Volume	Total cc. tenth-normal HCl	Highest concentration free HCl			1.1 to 1.9	2 to 2.9	Below 3	3.0 to 3.9	Below 4	4.0 to 4.9	Below 5	5.0 to 5.9	Below 6	6.0 to 6.9	Below 7	7.0 to 7.9	Below 8
8	52	44	90	58	82	1	180	0	0	0	0	0	0	0	0	0	7.8	7.8	92.2	100.0
9	74	64	285	199	84	1	100	0	16.0	16.0	14.0	30.0	26.0	56.0	14.0	70.0	30.0	100.0	0	100.0
10	56	44				2	245	36.7	2.0	38.7	0	38.7	0	38.7	4.1	42.8	57.2	100.0	0	100.0
11	44	36	220	185	100	2	328	24.4	20.1	44.5	6.1	50.6	11.0	61.6	23.2	84.8	15.2	100.0	0	100.0
12	68	56	125	80	80	2	360	7.2	25.5	32.7	18.3	51.0	10.6	61.6	21.7	83.3	13.9	97.2	2.8	100.0
13	60	44	167	103	100	1	182	24.2	3.3	27.5	25.3	52.8	6.6	59.4	37.3	96.7	3.3	100.0	0	100.0
14	80	56	270	160	70	2	362	39.3	17.1	56.4	10.5	66.9	9.4	76.3	9.9	86.2	13.8	100.0	0	100.0
15	90	84	130	101	96	2	240	21.7	40.8	62.5	10.8	73.3	9.2	82.5	16.7	99.2	0.8	100.0	0	100.0
16	74	64	105	99	104	1	180	57.8	14.4	72.2	2.2	74.4	6.7	81.1	7.8	88.9	10.0	98.9	1.1	100.0
17	78	68				1	180	76.7	10.0	86.7	3.3	90.0	7.8	97.8	2.2	100.0	0	100.0	0	100.0
18	68	58	190	162	106	1	165	54.5	36.4	90.9	0	90.9	0	90.9	0	90.9	6.1	97.0	3.0	100.0
19	58	50	430	304	110	2	240	93.8	0	93.8	0	93.8	0	93.8	0	93.8	6.2	100.0	0	100.0
20	74	68				2	316	74.8	17.7	92.5	5.7	98.2	0.6	98.8	0.6	99.4	0.6	100.0	0	100.0
Averages 9, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20						20‡	3078‡	43.12	18.30	61.42	8.75	70.17	7.99	78.16	12.13	90.29	9.08	99.37	0.63	100.0

\* Values in clinical units one hour after ingestion of meal.

‡ Totals for all thirteen subjects.

† Values obtained in the one-hour period after stimulation.

gastric secretion and little duodenal secretion. The gross appearance did not change for thirty-four minutes and during that time the pH values fluctuated between 1.30 and 2.20. The contents then intermittently contained more duodenal secretion and the pH values fluctuated wildly between 2.0 and 7.1 for fifty-six minutes, the pH values at the upper levels not lasting for longer than eight minutes at any one time. During the remainder of the observations the contents recovered appeared to be largely gastric juice and the pH values remained between 1.10 and 1.5 all the

time, with the exception of one period when the values rose to 6.25 for six minutes.

In this, as well as in other observations on patients with duodenal ulcer, the flow of acid gastric material from the tube was not always followed by a fairly prompt flow of amber colored fluid such as was usual in normal persons. In fact, the acid gastric juice was at times recovered almost unmixed with duodenal juice for fairly long periods. This means that the entrance of acid gastric material into the duodenum did not evoke the usual prompt secretory response on the part of

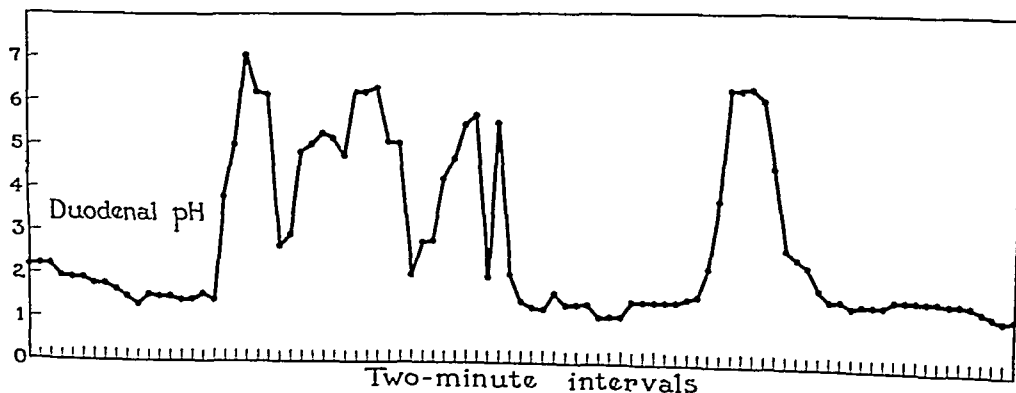


FIG. 2. THE TYPICAL BEHAVIOR OF VALUES FOR THE HYDROGEN ION CONCENTRATION OF THE DUODENAL CONTENTS OF PATIENTS WITH DUODENAL ULCER

the liver and pancreas that it did in normal individuals. The frequency and duration of the periods during which acid gastric juice was flowing from the duodenal tube varied considerably, just as in normal persons. However, the excursions to a pH value below 4 lasted longer than in normal subjects. Of the excursions below a pH value of 4, 53.4, 66.2 and 33.8 per cent lasted ten minutes or less, twenty minutes or less and twenty-one minutes or more, respectively. Excursions lasting twenty to thirty minutes occurred nine times, thirty to forty minutes four times, forty to fifty minutes five times, fifty to sixty minutes five times, seventy to eighty minutes twice, eighty to ninety minutes twice, 130 to 140 minutes once and 170 to 180 minutes once.

In some of the series of observations the duration of the periods when pH values were above 4 was so short that one might more properly speak of excursions above 4 than of excursions below 4. There was also a distinct tendency for the pH values to remain in the highly acid side of the range: thus, the pH values fell below 6, 70, 80 and 90 per cent of the time, respectively, in eleven, ten and six of the thirteen cases; below 4 for 50, 60 and 90 per cent of the time, respectively, in ten, seven and four of the thirteen cases studied.

A pH value as low as that seen for the acid gastric contents occurred in all but two subjects and for as much as 93.8 per cent of the time. A pH value below 4 occurred in all but Subject 8; the percentage of the duration of the observations during which pH values below 4 occurred varied from 30 to 98.

It appears that in a majority of cases of duodenal ulcer the fasting stomach pours acid into the duodenum during a high percentage of time, and that the outpouring of bile and pancreatic juice may be, but usually is not, in sufficient quantities to raise the pH values above 6. It also appears that the secretory response to the entrance of acid may be delayed so that there is little obvious effort on the part of the liver and pancreas to dilute and neutralize the acid for long periods. The duodenal contents remained highly acid a great part of the time.

The widely recognized variability of the response of the gastric secretory mechanisms to stimulation either with the carbohydrate water

meal or histamine may explain why no parallelism can be satisfactorily demonstrated in patients with duodenal ulcer between the degrees of secretory activity of the stomach and the behavior of pH values of the duodenal contents (Table II). Another possible explanation exists, namely, that the capacity for the neutralization of acid by the duodenum varies just as does the amount of acid entering the duodenum. Probably both variables influence the levels of hydrogen ion concentration.

#### SUMMARY AND CONCLUSIONS

The pH values of the duodenal contents removed through duodenal tube by continuous aspiration under negative pressure have been determined under fasting conditions in normal persons and in patients with duodenal ulcer. The pH values obtained in the fluid removed by tube may not be identical with those of the undisturbed contents because of the continuous removal of the duodenal contents and the shortened period of action of the acid gastric contents on the hormonal mechanisms, but the values may be used for comparative purposes since the same errors would exist in the data obtained from both normal persons and patients with duodenal ulcer.

It may be said that a rough parallelism exists between the degree of gastric secretory activity and the pH value of the duodenal contents of normal persons: the greater the secretory activity, the lower are the pH values. The same parallelism cannot be satisfactorily demonstrated in subjects with duodenal ulcer, a fact which suggests that there is a variability in the capacity of the duodenum of patients with duodenal ulcer to neutralize acid entering the duodenum.

The flow of a mixture of amber fluid and acid gastric contents from the tube usually is followed within a few minutes by an increased flow of amber fluid in normal persons, whereas this response is often delayed for varying lengths of time in patients with duodenal ulcer. This suggests that some disturbance of hormonal or chemical mechanism controlling the response of liver and pancreas to the entrance of acid gastric juice into the duodenum exists in some patients with duodenal ulcer.

The behavior of pH values in patients with duodenal ulcer contrasts with that seen in normal

persons. The pH values fell below 6, 4 and 2, respectively, 62, 18 and 3 per cent of the total duration of combined observations on normal persons whose gastric contents contained free hydrochloric acid and below 6, 4 and 2, respectively, 90, 70 and 43 per cent of the duration of combined observations on patients having ulcer in which there was evidence of entrance of acid gastric secretion into the duodenum. The percentage of time during which the pH values were below 4 (the point at which free hydrochloric acid becomes titrable) was four times and below 2 (values commonly seen in the acid gastric contents) twenty times as great in patients with duodenal ulcer as in normal individuals. Of the excursions of pH values below 4, 78, 100 and 0 per cent lasted ten minutes or less, twenty minutes or less, and twenty-one minutes or more, respectively, in normal persons with average gastric secretory activity, whereas 53, 66 and 34 per cent of the excursions of pH values below 4 lasted, respectively, the same length of time in patients with duodenal ulcer.

In short, the pH values were lower on the average, reached lower levels, and remained there for a longer period in patients with duodenal ulcer than in normal persons. The duodenal mucosa of patients with duodenal ulcer was bathed in a more highly acid fluid for longer periods than in normal persons.

The more highly acid conditions of the duodenal contents in patients with duodenal ulcer appear to be due (1) to larger quantities of acid gastric juice entering the duodenum; (2) to a relative, if not an absolute deficiency of neutralizing and diluting fluid, and (3) to a disturbance of neutralizing and diluting mechanism indicated by delayed appearance of amber colored duodenal contents following the entrance of acid gastric contents into the duodenum.

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# A STUDY OF URINARY RIBOFLAVIN EXCRETION IN MAN<sup>1</sup>

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The occurrence of riboflavin deficiency in man has been described by several investigators (1 to 5). Since urinary excretion tests have proved to be of considerable value in the diagnosis of thiamin and ascorbic acid deficiencies, the present study was undertaken to determine the value of such tests in the diagnosis of riboflavin deficiency.

A pronounced decrease in the urinary riboflavin excretion has been observed in rats and dogs maintained on riboflavin-low diets (6, 7, 8). Spies, Bean and Ashe (9) have reported a decrease in the amount of riboflavin present in the urine of pellagrins with riboflavin deficiency, and Emmerie (10) has observed that urinary riboflavin excretion of a subject on a diet restricted in riboflavin was 43 to 60 per cent of that observed when the subject was on a normal diet. Both groups of investigators (9, 10) noted that the administration of riboflavin was followed by a rapid excretion of the vitamin in the urine. Neuweiler (11) reported a case of ariboflavinosis in an infant who completely retained large doses of riboflavin.

## MATERIAL AND METHODS

Five patients in the Nutrition Clinic of the Hillman Hospital, Birmingham, Alabama, were chosen for this study. This group (Cases I, II, III, IV and V) consisted of malnourished persons who were suffering from a multiple vitamin deficiency. They were hospitalized throughout the study and were restricted to diets deficient in various members of the vitamin B-complex during the course of the investigation.<sup>2</sup> Two patients (Cases VI and VII) from the Hillman Hospital were

used as controls and were kept on the regular hospital diet. Case VI was convalescing from a severe burn, and Case VII had recently recovered from an attack of empyema. Both were in a good state of nutrition during the observation period.

Twenty-four-hour urine specimens were collected from each patient in dark bottles containing 1 cc. of glacial acetic acid and a small amount of toluene. The volume was measured and aliquots were brought to pH 6.6 and stored in the refrigerator under toluene. Samples were collected over a period of two to four weeks.

The riboflavin content of the urine was determined by the microbiological method of Snell and Strong (12). The reliability of this method for the determination of both the free and combined forms of riboflavin in a number of biological fluids has been adequately established (12, 13). When necessary, the urine was diluted with distilled water in order to adjust the riboflavin content to the correct assay range (approximately 0.1 microgram of riboflavin per ml.). All assays were made at three different levels, each level being run in duplicate. It was found that the sample of urine could be stored in the refrigerator for a period of two weeks with no loss in the riboflavin content.

The "saturation" tests were performed by administering riboflavin through intravenous injection and determining the riboflavin content of the following twenty-four-hour urine specimens. Two hundred micrograms of riboflavin per kilogram of body weight were given to all patients except Case I who received 400 micrograms per kilogram of body weight. In order to study the rapidity with which the riboflavin was excreted after injection, urine specimens were taken at varying intervals in the case of Subjects I, II, III and IV.

## RESULTS

The average daily riboflavin excretion for the patients maintained on the deficient diets was as follows: Case I, 59 micrograms; Case II, 61 micrograms; Case III, 58 micrograms; Case IV, 91 micrograms and Case V, 65 micrograms. The values ranged between 58 and 91 micrograms. The variation from day to day was small. For example, the daily riboflavin excretion of Case I varied from 53 to 69 micrograms over a period of one week. It is evident that the riboflavin ex-

<sup>1</sup> These studies were aided by grants from the John and Mary R. Markle Foundation and the Wisconsin Alumni Research Foundation.

<sup>2</sup> The diets employed had the following compositions:  
Diet A. Hominy grits, cornmeal, white flour, sugar, pork fat, sweet potato, cabbage, spinach, furnishing approximately 300 micrograms of riboflavin per 2,000 calories.  
Diet B. Hominy grits, white flour, cornmeal, French bread, Karo syrup, sugar, black-eyed peas, salt pork, lard, tomato juice, furnishing negligible amounts of riboflavin.



cretion of these patients was very low and distinctly lower than that of Case VI and VII on the regular hospital diet who excreted 236 and 270 micrograms, respectively. The riboflavin excretions for these normal patients were also somewhat low since Strong *et al* (14) found the daily riboflavin excretion for twelve normal subjects to range from 477 to 835 micrograms, with an average value of 625 micrograms. The most logical explanation for the rather low values for the two normal cases in this study is that the total food intake of these patients during hospitalization was considerably lower than that of the normal and active individuals studied by Strong *et al* (14). It is our belief, therefore, that the twenty-four-hour urinary riboflavin excretion values may be used as an indication of the dietary intake of riboflavin. Some idea of the degree of deficiency may be obtained by following the daily excretion of riboflavin for several consecutive days. These results are in agreement with those of Ferrebee (15).

The results of the "saturation" tests are given in Table I. No correlation could be found between the daily urinary excretion of riboflavin and the degree of retention of a given test dose of riboflavin. Thus Case V, excreting 65 micrograms of riboflavin per day before treatment, retained the same percentage of administered riboflavin as did Case VI who was excreting 236 micrograms of riboflavin per day. Similarly, Cases I, II, III and IV, with a very low daily urinary excretion of riboflavin, retained amounts

of a given test dose of riboflavin which were of the same order as those retained by two normal subjects studied by Strong *et al* (14).

The rapidity with which riboflavin given by intravenous injection is excreted in the urine is shown in Table II. Thirty to 40 per cent of the administered riboflavin was excreted in the urine within one hour after injection in the three subjects who received 200 micrograms of riboflavin per kilogram of body weight. Forty-two per cent of the injected riboflavin was excreted within three hours in the subject who received 400 micrograms of riboflavin per kilogram of body weight.

TABLE II  
The rate of excretion through the urine of intravenously administered riboflavin

Case	Riboflavin given by intravenous injection	Micrograms of riboflavin excreted in the urine within the stated time intervals following the injection*									
		15	30	60	2	3	4	5	6	7	8
	micrograms	minutes			hours						
I	21800†		1660	3980		9260		11570		12350	13020
II	14000‡	1890		6240		8452		6665		6773	7151
III	12200‡			4110	4346		4369		4415		4548
IV	11200‡			4400	5255		5903		6023		6021
											6995

\* Every value represents the total amount of riboflavin which has been excreted up to the given time following injection.

† Equivalent to 400 micrograms of riboflavin per kilogram of body weight.

‡ Equivalent to 200 micrograms of riboflavin per kilogram of body weight.

## DISCUSSION

The lack of correlation between the daily urinary excretion of riboflavin and the degree of retention of administered riboflavin makes it evident that the riboflavin "saturation" test, as carried out under our conditions, has but little diagnostic value in subjects with a riboflavin deficiency which is complicated with other vitamin deficiencies.

The results of the "saturation" tests obtained with the human subject are in contrast with those obtained with the dog. An uncomplicated riboflavin deficiency can be produced in the dog and it has been found in this species that the degree of retention of a test dose of riboflavin administered either orally or subcutaneously is a measure of the severity of the riboflavin deficiency (8).

TABLE I

The effect of a diet low in riboflavin upon the daily urinary excretion of riboflavin and upon the retention of intravenously administered riboflavin

Case	Average daily excretion of riboflavin in the urine	Riboflavin given by intravenous injection*	Percentage of the test dose of riboflavin excreted in the urine
	micrograms	micrograms	per cent
I	59	21800	72
II	61	14000	51
III	58	12200	37
IV	91	11200	63
V	65	12700	10
VI	236	11800	10
VII	270		

\* These amounts of riboflavin are equivalent to 400 micrograms per kilogram of body weight in Case I and 200 micrograms per kilogram of body weight in the other subjects.

Therefore, a dog suffering from a riboflavin deficiency retains practically all of the administered riboflavin, while a normal dog excretes a significantly larger percentage of the corresponding dose of riboflavin. The test doses of riboflavin used in the dog experiments were similar to those employed in the human studies. It should be emphasized that the "saturation" tests were performed on dogs with an uncomplicated riboflavin deficiency, while the human subjects in this study were suffering from a multiple vitamin deficiency. Thus further work is indicated in order to evaluate the effect of other nutritional factors upon the retention of a given test dose of riboflavin.

#### SUMMARY

1. The daily urinary riboflavin excretion of five patients consuming riboflavin-low diets and two patients on a hospital diet was determined. The results of these cases, together with those studied by Strong *et al* (14), indicate that there is a marked variation in the daily urinary riboflavin excretion which is correlated with the dietary intake of riboflavin. Thus the determination of the daily urinary riboflavin excretion serves as an aid in judging the degree of riboflavin deficiency.

2. Riboflavin "saturation" tests, employing intravenous injections of 200 and 400 micrograms of riboflavin per kilogram of body weight, were carried out on six subjects. No correlation between the amount of the test dose of riboflavin retained and the daily urinary riboflavin excretion was obtained. Therefore, no diagnostic value could be attached to the "saturation" tests performed on the subjects of this study.

3. The rate of excretion of riboflavin into the urine after injection was determined. A very rapid loss in the urine was observed.

#### CASE ABSTRACTS

*Case I.* A 39-year-old white male had been seen in 1939 with pellagra. When admitted to the hospital April 29, 1940, he complained of burning of the stomach, dizziness, sore lips and tongue, and scaling, red ulcerated skin on hands, forearms and legs. He was chronically addicted to alcohol and his diet at home consisted of fat meat, corn bread, syrup, dried beans and fruit, and a glass of milk per day. His tongue was red at the tip and the skin on his face and neck was rough and pigmented; on his forearms, hands, feet and legs there was a dry, scaly, pigmented dermatitis with ulcerated areas

near the center of the lesions. He had tender calves, absent knee jerks and ankle jerks and absent appreciation of vibration below the hips. The diagnosis was multiple deficiency of vitamins of the B-complex, principally nicotinic acid and thiamin. During this investigation his vitamin intake was controlled by Diet A.

*Case II.* A 43-year-old white male was admitted to the hospital because of nervousness and pain in the epigastrium. Symptoms began March, 1940. He became dizzy, had frequent headaches, burning of the eyes, tongue and lips. He had lost his appetite and had alternate constipation and diarrhea. His diet at home consisted of a glass of milk daily, pork, dried fruit and vegetables, white bread, karo and butter. Both the bulbar and tarsal conjunctivae were extremely injected and his eyes watered continuously. His lips and buccal mucous membranes were red. There were superficial, dry ulcerations at the angles of the mouth, and his tongue was red at the tip. There was mild tenderness in the epigastrium, his calves were tender to pressure and his ankle jerks were absent. The diagnosis was multiple deficiency of vitamins of the B-complex, principally nicotinic acid, riboflavin and thiamin. During his hospital stay he was kept on control Diet B.

*Case III.* A 27-year-old, colored female entered the hospital complaining of sore mouth and pain in the legs of two months' duration. At the beginning of her illness she had had scaly, red dermatitis on her fingers and hands but this had cleared. She had had frequent attacks of diarrhea, vomiting, sleeplessness, nervousness, and burning of the mucous membranes of her mouth. Her diet consisted of dried fruit and vegetables, corn bread, biscuits, fat meat and one egg per day. Her conjunctivae were injected and inflamed; her tongue was slick and red; the mucous membranes at the angles of the mouth showed atrophic changes frequently seen after recently healed cheilosis. The skin of the forearms, hands and fingers were hyperpigmented and rough. Her calves and the soles of her feet were tender; ankle jerks were absent, and the skin of her feet and ankles was hyperesthetic to stroke. The diagnosis was multiple deficiency of vitamins of the B-complex, principally nicotinic acid, thiamin and riboflavin. During this investigation she was maintained on control Diet A.

*Case IV.* A 53-year-old; white male entered the Hillman Hospital with dermatitis on the arms and hands. He complained of headache, dizziness, ringing in the ears, loss of appetite, and burning and cramping of the legs. He was chronically addicted to alcohol and his usual home diet consisted of three eggs per day, fat meat, dried fruits and vegetables, white bread, butter and desserts. His conjunctivae, particularly on the lower lids, were inflamed and granular in appearance. His tongue was coated, and there were scars surrounded by mild erythema at the angles of the mouth, suggesting healing cheilosis. The skin was red, rough and desquamating. There was a sharp border of demarcation between the



TABLE II

*Sera from cases of infectious mononucleosis. Sheep cell agglutination tests*

Serum	Serum dilution . . . .		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	Titer (Final dilution)	Wasser- mann	Hinton
	Final dilution . . . .		1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024			
	Day of dis- ease	Severity											
D. B.	4	Severe	+++	0	0	0	0	0	0	0	1:8	0	0
D. B.	8		++++*	++++	++++	++++	++++	0	0	0	1:128	0	0
D. B.	15		++++	++++	++++	++++	++++	++	+	+	1:1024	0	0
D. B.	21		++++	++++	++++	++++	++++	++	+	0	1:512	0	0
D. B.	37		++++	++++	++++	++++	+	0	0	0	1:128	0	0
A. G.	15	Severe	++++	++++	++++	++++	++++	++	0	0	1:256	0	0
A. G.	25		++++	++++	++++	++++	++++	+	0	0	1:256	0	0
A. G.	31		++++	++++	++++	++++	++++	++	0	0	1:256	0	0
A. G.	65		++++	++++	++++	++++	++	0	0	0	1:128	—	0
M. L. R.	5	Moderate	++++	++++	++++	++++	++++	++	++	+	1:1024	0	0
M. L. R.	17		++++	++++	++++	++++	++++	++++	++	+	1:1024	0	0
M. L. R.	45		++++	++++	++++	++++	++++	++	+	0	1:512	0	0
A. H.	8	Moderate	++++	++++	++++	++++	++++	++++	++	0	1:512	0	0
A. H.	15		++++	++++	++++	++++	++++	++++	++++	++++	1:1024+	0	0
C. M.	15	Moderate	++++	++++	++++	++++	++++	++++	++++	++++	1:1024+	0	0
C. M.	23		++++	++++	++++	++++	++++	++++	++++	++++	1:1024+	0	0
O. S.	14	Mild	++++	++++	++++	++++	++++	++++	+++	+	1:1024	0	0
R. S.	21	Mild	++++	++++	++++	++++	++++	+++	+++	+	1:1024	0	0
A. L.	10	Questionable	++++	++++	++++	++	0	0	0	0	1:64	0	0
A. L.	15		++++	++++	++++	++	++	0	0	0	1:128	0	0
P. M.	15	Mild	++++	++++	++++	++++	++++	+++	++	++	1:1024	0	0
R. B.	9	Questionable	++	0	0	0	0	0	0	0	1:8	0	0
D. M.	10	Mild	++++	++++	++++	++++	++++	++++	++++	++++	1:1024+	0	0
A. G.	?	Questionable	++++	++++	++++	++++	++++	++++	++	0	1:512	0	0
M. L.	24	Very severe	—	++++	++++	++	++	++	+	0	1:512	0	0
M. L.	82		++++	++++	++++	++	+	0	0	0	1:128	—	—

\* Represents very strong reaction.

noted that most of these fall within the normal range given by most authors, namely 1:64 (final dilution) or less. The three exceptions occur in the sera of patients who had received horse serum for the treatment of pneumonia, and the highest titer, 1:1024, was recorded in a patient with serum disease. It has been known for some time that the injection of horse serum would lead to the production of "heterophile" antibodies. Davidsohn (13) has shown that these may be distinguished from the sheep cell agglutinins of infectious mononucleosis by their disappearance following absorption of the serum with fresh guinea pig kidney.

In Table II are recorded the results of sheep cell agglutination, Wassermann, and Hinton tests on 13 cases of clinically diagnosed infectious mononucleosis. In this group no false positive serological reactions for syphilis were noted, al-

though the last patient, M. L.,<sup>3</sup> had a positive spinal fluid Hinton when the blood Hinton was negative. All but 1 case, R. B., in whom the diagnosis was questionable, showed a marked elevation of the titer of sheep cell agglutinins. D. B., our most adequately studied case, was followed from the fourth day until complete recovery occurred. In his serum the titer was observed to rise from a normal level of 1:8 on the fourth day to a peak of 1:1024 on the fifteenth day, and then gradually to fall back toward normal. These 13 cases represent a fair cross section of the disease, with the severity varying markedly. In accordance with the observations of others, there was no correlation between the severity of the disease and the titer of sheep cell agglutinins attained.

<sup>3</sup> The authors wish to express their appreciation to Dr. Ruth B. Thomas, Dover-Foxcroft, Maine, for sending the serum samples from this patient.

TABLE III  
Control sera. *Listerella agglutination*

Serum dilution.....	1:10	1:20	1:40	1:80	1:160	1:320	Titer (Final dilution)	
Final dilution.....	1:20	1:40	1:80	1:160	1:320	1:640		
Serum							vs. C.H.	vs. U.M.
L. H.....	0* 0†	0 0	0 0	0 0	0 0	0 0	0	0
P. B.....	+ +++	0 ++	0 0	0 0	0 0	0 0	1:20	1:40
J. W.....	± +	0 0	0 0	0 0	0 0	0 0	0	1:20
G. D.....	+++ 0	++ 0	0 0	0 0	0 0	0 0	1:40	0
C. J.....	0 ++	0 ±	0 0	0 0	0 0	0 0	0	1:20
M. R.....	++++ +	+++ 0	+ 0	0 0	0 0	0 0	1:80	1:20
W. S.....	++++ ++++	++ ++	0 0	0 0	0 0	0 0	1:40	1:40
M. G.....	+ +	0 0	0 0	0 0	0 0	0 0	1:20	1:20
D. G.....	± +	0 0	0 0	0 0	0 0	0 0	0	1:20
W. F.....	0 ++	0 0	0 0	0 0	0 0	0 0	0	1:20
A. F.....	0 0	0 0	0 0	0 0	0 0	0 0	0	0
W. H.....	0 0	0 0	0 0	0 0	0 0	0 0	0	0
M. B.....	± +++	0 +	0 0	0 0	0 0	0 0	0	1:40
E. W.....	+ ++++	0 ++	0 0	0 0	0 0	0 0	1:20	1:40
A. R.....	++ +	0 0	0 0	0 0	0 0	0 0	1:20	1:20
J. DeM.....	+ ++	+ +	0 0	0 0	0 0	0 0	1:40	1:40
M. W.....	0 +++	0 ±	0 0	0 0	0 0	0 0	0	1:20
W. McK.....	± +++	0 ±	0 0	0 0	0 0	0 0	0	1:20
S. M.....	+++ 0	0 0	0 0	0 0	0 0	0 0	1:20	0
J. S.....	± ++	0 0	0 0	0 0	0 0	0 0	0	1:20
Pooled immune rabbit serum.....	++++ ++++	++++ ++++	++++ ++++	++++ ++++	++++ ++++	++ ++++	1:640	1:640+

\* Upper line = vs. C. H.

† Lower line = vs. U.M.

Tables III and IV record the results of agglutination tests with representative strains of the two serological groups of the genus *Listerella*. The data require little comment. The recorded titers are slightly higher for the cases of infectious mononucleosis than for the controls. No definite trends are observed in any particular case, the variations between samples being within the limits of error, with the possible exception of the last case, M. L. However, in her serum the titer for *C. H.* rose slightly, while that for *UM.* fell during recovery.

## DISCUSSION

This study was undertaken in order to see whether patients with infectious mononucleosis develop agglutinins for either of the two known

serological groups of the genus *Listerella*. Although we have observed slightly higher agglutinin titers in the sera of infectious mononucleosis patients than in the sera of normal people, or of those suffering from other diseases, the interpretation of this finding is difficult. The numbers in either series are not sufficiently large to eliminate the possibility that chance variation may account for the observed differences. It might be an example of the anamnestic reaction, but many of the control sera were taken from patients with acute illnesses, and the sera of infectious mononucleosis patients failed to show a reduction in titer when they were obtained during convalescence.

In view of Kolmer's (8) results, which we have confirmed, (9) the increased agglutination of *Listerella* cannot be due to the presence of

TABLE IV  
*Sera from cases of infectious mononucleosis. Listerella agglutination*

Serum	Serum dilution		1:10	1:20	1:40	1:80	1:160	1:320	Titer (Final dilution)	
	Final dilution		1:20	1:40	1:80	1:160	1:320	1:640		
	Day of disease	Severity							Vs. C.H.	Vs. UM.
D. B.	4	Severe	++* +++†	0 ++	0 0	0 0	0 0	0 0	1:20	1:40
	8		+ +++	0 ++	0 0	0 0	0 0	0 0	1:20	1:40
	15		+ +++	0 ++	0 ±	0 0	0 0	0 0	1:20	1:40
	21		± +++	0 +	0 0	0 0	0 0	0 0	0	1:40
	37		+ +++	0 ++	0 0	0 0	0 0	0 0	1:20	1:40
A. G.	15	Severe	+ ++	0 0	0 0	0 0	0 0	0 0	1:20	1:40
	25		± +	0 0	0 0	0 0	0 0	0 0	0	1:40
	31		+ ++	0 ±	0 0	0 0	0 0	0 0	1:20	1:40
	65		± ++	0 0	0 0	0 0	0 0	0 0	0	1:40
M. L. R.	5	Moderate	+ ++	0 +	0 0	0 0	0 0	0 0	1:20	1:40
	17		+ +++	0 +	0 0	0 0	0 0	0 0	1:20	1:40
	45		± ++	0 ±	0 0	0 0	0 0	0 0	0	1:20

\* Upper line = vs. C. H.

† Lower line = vs. UM.

TABLE IV—Continued

Serum	Serum dilution		1:10	1:20	1:40	1:80	1:160	1:320	Titer (Final dilution)	
	Final dilution		1:20	1:40	1:80	1:160	1:320	1:640		
	Day of disease	Severity							Vs. C.H.	Vs. U.M.
A. H.	8	Moderate	++ ++++	± +	0 +	0 0	0 0	0 0	1:20	1:80
	15		++++ +++++	+ ++++	0 ++	0 0	0 0	0 0	1:40	1:80
C. M.	?15	Moderate	++++ ++++	+ +	0 0	0 0	0 0	0 0	1:40	1:40
	?23		++ ++	0 ±	0 0	0 0	0 0	0 0	1:20	1:20
O. S.	?14	Mild	--- +++++	--- ++++	--- +	--- 0	--- 0	--- 0	---	1:80
R. S.	?21	Mild	+++ ++++	± ±	0 0	0 0	0 0	0 0	1:20	1:20
A. L.	10	Questionable	+ ++	+ 0	0 0	0 0	0 0	0 0	1:40	1:20
	15		+ ++	0 +	0 0	0 0	0 0	0 0	1:20	1:40
P. M.	?15	Mild	+++ ++++	+ ++++	0 +	0 0	0 0	0 0	1:40	1:80
R. B.	9	Questionable	+ ++	0 ±	0 0	0 0	0 0	0 0	1:20	1:20
D. M.	10	Mild	+++ ++++	++ ++++	0 ++	0 0	0 0	0 0	1:40	1:80
A. G.	?	Questionable	++++ ++++	++++ ++	+ +	0 0	0 0	0 0	1:80	1:80
M. L.	24	Very severe	--- ---	+++ ++++	++ ++++	+ ++++	± +	0 0	1:160	1:320
	82		--- ---	+++ ++++	+++ ++	++ +	± 0	0 0	1:160	1:160

sheep cell agglutinin, since anti-*Listerella* sera do not agglutinate sheep cells, nor do high titer anti-sheep cell sera agglutinate *Listerella*.

Perhaps the strongest point against the significance of the increased titer found in the mononucleosis patients is the lack of any definite trend either upward or downward during the progression of the disease and recovery from it.

Unfortunately, we did not have access to Nyfeldt's organisms obtained from what seem to be proven cases of infectious mononucleosis, so that we do not know whether they represent a different serological group. If this were the case, a distant antigenic relationship might explain the slight increase in titer found with our strains. However,

we received Julianelle's<sup>4</sup> type strains, one of which was obtained from the blood of a mononucleosis patient, and they fall into the two known groups so that this seems unlikely. One is forced to conclude that, if infectious mononucleosis is caused by a member of the genus *Listerella*, the organisms remain localized, producing an early slight rise in agglutinin titer, and persist for long periods of time, thus maintaining this titer long into convalescence. The latter is not impossible, in view of the prolonged, insidious course of the disease in many patients. However, it is evident

<sup>4</sup>The authors wish to express their appreciation to Dr. L. A. Julianelle for sending these strains and for making his results available to us before their publication.

that unless the interpretation of our data is considerably strained, there is no conclusive serological evidence for an etiological relationship between the known strains of *Listerella* and infectious mononucleosis.

Since the completion of this work, a report by Julianelle (14) has appeared which, in a larger series of cases, records results similar to ours. He failed to grow *Listerella* from the blood in 17 out of 18 patients with infectious mononucleosis, the one exception being the case previously reported. We have studied throat cultures from 6 patients, blood cultures from 5 acutely ill patients, and a lymph node from a patient in the florid phase of the disease with a sheep cell agglutination titer of 1:512, and have never isolated any organisms resembling *Listerella*. Julianelle also reported finding a slightly elevated *Listerella* agglutination titer in 13 out of 35 patients with mononucleosis.

#### SUMMARY

1. The sera of 5 normal adults, 15 patients with miscellaneous diseases, and 13 patients with infectious mononucleosis, were tested for agglutinins against sheep cells and a representative strain of each of the two known serological groups of the genus *Listerella* (*Bact. monocytogenes*).

2. The results of the sheep cell agglutination tests were in accordance with the findings of many previous workers.

3. The results of the *Listerella* agglutination tests showed a slightly elevated titer in the sera of the patients with infectious mononucleosis as compared to the control sera.

4. There was no significant trend upward or downward in the *Listerella* agglutinin titer during the course of the disease or recovery from it.

5. It is concluded that this study does not suggest any definite etiological relationship between

the known *Listerella* organisms and infectious mononucleosis.

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# THE CEPHALIN-CHOLESTEROL FLOCCULATION TEST AS AN AID IN THE DIAGNOSIS OF HEPATIC DISORDERS<sup>1</sup>

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Clinically, it is difficult and often impossible to diagnose accurately the type of hepatic or biliary tract disorder in a jaundiced individual. The determination of the prognosis in these patients is likewise difficult. It is in this particular field that the available laboratory aids are least satisfactory. The lack of simple, sensitive, and yet specific methods for measuring the multiple functions attributed to the liver has notably contributed to the problem.

Recent investigations by Hanger (1, 2) indicate that emulsions prepared from mixtures of sheep brain cephalin and cholesterol are flocculated by serum from jaundiced patients with active disturbances of the liver parenchyma. This serological reaction was used to differentiate hepatogenous from obstructive jaundice, since it was positive in the former and negative in the latter type. Hanger concluded that the test was more sensitive in the detection of liver disturbances than the commonly employed functional tests. Serial determinations proved to be of aid in estimating the degree and persistence of the disorder. Although the mechanism of the cephalin-cholesterol flocculation reaction is not clearly understood, Hanger (2) suggests that it depends upon the capacity of an altered serum globulin to become affixed to the colloidal elements of the emulsion.

The purpose of the present investigation was to observe the cephalin-cholesterol flocculation reaction with sera from patients having a variety of hepatic and biliary tract disorders, and to compare these results with the clinical course and other laboratory data.

## METHODS

*Preparation of stock solution.* The cephalin employed in the preparation of the stock reagent was prepared from fresh sheep brains in the manner outlined by Chargaff (3), except that the acetone extraction was carried

out 6 times instead of 3 to assure thorough dehydration. Approximately 750 mgm. of desiccated material rich in cephalin was obtained from a sheep brain of average size. The dehydrated material was immediately mixed with cholesterol and the stock ether solution prepared exactly as previously described (2). This reagent can be kept without deterioration in a glass-stoppered bottle in an ice box at 5° C. for at least one year.

*Technique for flocculation test.* The cephalin-cholesterol test was performed in the manner described elsewhere (2). One cc. of the stock ether solution is slowly added with stirring to 35 cc. of distilled water. The mixture is warmed to from 65 to 70° C., slowly heated to boiling, and then permitted to simmer until the final volume is reduced to exactly 30 cc. All traces of ether are removed in this manner. After cooling to room temperature, 1 cc. of the milky, translucent emulsion is added to a centrifuge tube containing 0.2 cc. of the patient's serum and 4 cc. of freshly prepared 0.85 per cent sodium chloride solution. The mixture is thoroughly shaken, the tube stoppered with cotton, placed upright in a rack, and allowed to stand undisturbed at room temperature for 24 hours. At the end of this period notation as to the presence or absence of flocculation is made. With normal human sera the emulsion persists as a stable homogeneous suspension. Pathological serum flocculates the lipid material which tends to settle to the bottom of the tube. The positive reactions are graded +, ++, +++, or +++++, depending on the amount of precipitation and clearing in the upper portion of the suspension. As previously reported (2), few precautions are necessary. The present observations indicate that the serum should not be drawn more than 6 hours prior to the performance of the test.

*Material studied.* Sera from 284 normal individuals, from 455 patients without evidence of liver or biliary tract disease, and from 141 patients with proven liver or biliary tract disease were studied in the manner described. All but 5 of the patients were adults. Each patient in the latter 2 groups was hospitalized throughout the period of observation; the average period of observation was 12.8 days. The cephalin-cholesterol flocculation test was performed at least twice a week on each of the patients. A control test was done on a normal serum on every occasion.

The diagnosis was established in many of the 141 patients with liver or biliary tract disease by histopathological studies. In the remainder of the cases the diagnosis depended upon the clinical findings and course, and the laboratory data. Abdominal surgery was done on 72

<sup>1</sup>This study was aided in part by a grant from the Wisconsin Alumni Research Foundation.

patients and in 14 instances a section of the liver was obtained for biopsy. Complete autopsy was performed on 32 of the 40 patients with liver or biliary tract disease who died. Blood, urine, and stool examinations were done routinely. The urobilinogen in the urine of all jaundiced patients was determined quantitatively on repeated occasions. The icterus index and plasma prothrombin (4) were determined each time that a flocculation test was done. The serum albumin and globulin were measured at least once in 88 of the 141 patients with liver or biliary tract disease. Additionally, the galactose tolerance test and the hippuric acid synthesis and excretion test were frequently employed.

### RESULTS

*Flocculation test in normal individuals or patients without evidence of liver or biliary tract disease.* The flocculation reaction was tested on sera obtained from 284 normal individuals and in no instance did significant flocculation occur. The test was also performed on the sera from 455 patients with a variety of diseases, none of whom showed definite evidence of a liver or biliary tract disturbance. In this latter group a positive reac-

tion was noted in 15 individuals. The diagnoses in these 15 cases were as follows: central nervous system syphilis under treatment with malaria (3 cases), pneumonia (2 cases), thyrotoxicosis (2 cases), and one case each of pelvic inflammatory disease, nephrosis, pyelonephritis, septic arthritis, scarlet fever, acute tertian malaria, pemphigus, and paroxysmal nocturnal hemoglobinuria. In several of these 15 patients there might well have been an associated liver disturbance but confirmatory data were lacking.

The remainder of this communication will be limited to a report of the observations on the group of 141 patients with hepatic or biliary tract disease. For convenience in obtaining a comparative analysis, these cases have been divided into 5 groups, as follows: (1) cholecystitis without hepatic disease, (2) acute or subacute hepatitis, (3) cirrhosis of the liver, (4) obstructive jaundice, and (5) focal lesions of the liver.

*Flocculation reaction in cholecystitis without hepatic disease.* Thirty-four patients with chronic

TABLE I  
*Flocculation reaction in 36 patients with acute or subacute hepatitis*

Case	Initials	Sex	Age	Diagnosis	Basis for diagnosis	Cephalin-cholesterol flocculation	Ic-terus index	Plasma prothrombin	Hip- puric acid test	Comments
			years					per cent	per cent	
1	M. Q.	F	41	Acute hepatitis	Clinical	+++	20	60		Recovery after 1 month.
2	C. H.	F	49	Acute hepatitis	Clinical	++	8	100	86	Recovery after 2 weeks.
3	M. W.	F	14	Acute hepatitis	Clinical	++++	20	60	0	Died 3 weeks after onset.
4	F. H.	F	50	Acute hepatitis	Clinical	++	12	100	130	Recovery after 3 weeks.
5	D. R.	F	19	Acute catarrhal jaundice	Clinical	++++	15	70	52	Typical course with recovery.
6	E. E.	F	34	Acute catarrhal jaundice	Clinical	+++	22	45	35	Typical course with recovery.
7	J. V.	M	32	Acute catarrhal jaundice	Clinical	++	15	100	89	Typical course with recovery.
8	J. B.	M	23	Acute hepatitis (postarsphenamine)	Clinical	++++	60	50	12	Recovery after 5 months.
9	H. A.	F	33	Acute hepatitis (postarsphenamine)	Clinical	++++	65	25	0	Recovery after 2 weeks.
10	D. T.	F	39	Acute hepatitis (postarsphenamine)	Clinical	++++	15	65	0	Recovery after 1 week.
11	M. S.	F	42	Acute hepatitis (following divinyl ether anesthesia)	Clinical	++++	50	70	18*	Recovery after 6 months.
12	F. R.	F	65	Acute hepatitis (following ruptured appendix)	Autopsy	+++	30	30	10*	
13	A. S.	M	57	Acute hepatitis (following ruptured gall bladder)	Operation	+++	30	100	27*	Surgical drainage. Recovered.
14	L. W.	F	20	Hyperemesis gravidarum	Clinical	++++	15	17	48*	Recovered. Normal delivery.
15	C. S.	F	22	Hyperemesis gravidarum	Clinical	+++	18	50	37*	Recovered. Normal delivery.
16	C. S.	F	34	Acute hepatitis. Tuberculosis	Clinical	++	15	65		Still in sanatorium.
17	E. H.	F	27	Acute hepatitis. Congenital syphilis	Biopsy of liver	++++	8	20	21	Improved.
18	F. T.	F	59	Subacute hepatitis	Biopsy of liver	+++	5	65	51	Improved. Macrocytic anemia.
19	M. S.	F	45	Subacute hepatitis	Biopsy of liver	++	8	70	80	Apparent recovery.
20	M. J.	F	39	Subacute hepatitis	Clinical	++	12	100	115	Apparent recovery.
21	K. L.	F	59	Subacute hepatitis. Cholelithiasis	Biopsy of liver	++	6	100	110*	Cholecystectomy.
22	L. R.	F	38	Subacute hepatitis. Cholelithiasis	Operation	+++	15	100	93	Cholecystectomy.
23	E. H.	F	62	Subacute hepatitis. Cholelithiasis	Operation	+	15	100		Cholecystectomy.
24	E. O.	F	41	Subacute hepatitis. Cholelithiasis	Biopsy of liver	0	3	80	90	Cholecystectomy.
25	C. B.	F	54	Subacute hepatitis. Cholelithiasis	Biopsy of liver	0	50	50	48	Cholecystectomy.
26	S. R.	F	39	Subacute hepatitis. Cholelithiasis	Operation	++	25	50		Cholecystectomy.
27	C. D.	M	55	Chronic passive congestion of liver (red atrophy)	Autopsy	+++	5	100		
28	C. S.	M	22	Chronic passive congestion of liver (red atrophy)	Autopsy	++++	25	25		
29	F. V.	M	54	Chronic passive congestion of liver (red atrophy)	Autopsy	++++	10	100	65	
30	H. McL.	M	63	Chronic passive congestion of liver	Autopsy	+++	5	70	62	
31	F. W.	M	59	Chronic passive congestion of liver	Autopsy	+++	75	55	0	
32	C. A.	M	39	Chronic passive congestion of liver	Autopsy	++++	20	15		
33	G. B.	M	30	Chronic passive congestion of liver	Clinical	+++	15	45	54	Still under observation.
34	F. D.	F	70	Chronic passive congestion of liver	Clinical	++	3	100		Still under observation.
35	S. W.	F	65	Chronic passive congestion of liver	Clinical	++++	40	10	0	Hemorrhagic diathesis. Died.
36	A. M.	F	41	Chronic passive congestion of liver	Clinical	++	5	65		No further observations.

\* Denotes intravenous administration of sodium benzoate.

TABLE II

*Changes in the cephalin-cholesterol flocculation reaction and other laboratory data during the course of an acute toxic hepatitis (Case 1, Table I)*

Date	Cephalin-cholesterol flocculation	Icterus index	Urobilinogen in urine	Plasma prothrombin	Hippuric acid test	Serum proteins		Comments
						Albumin	Globulin	
1939								
			mgm. per 100 cc.	per cent	per cent	grams per 100 cc.	grams per 100 cc.	
November 2	+++	20		60				Onset of painless jaundice. Anorexia. Fever.
4	++++	45		60				
7	+++	60		32				
10	++++	50			0 (oral)			
15	++	60	7.3	20	0 (oral)	4.1	2.6	Extremely ill. Fever continues. Clinical improvement. Afebrile. Feels well.
17	+	40		25				
21			1.2		65 (oral)	3.9	2.4	
22	+	20		45				
25	0							
December 2	0	20	less than 0.9	60	78 (intravenous)	4.3	2.1	
6	0	8		100				
8	0	4	less than 0.9	100	.			Discharged from hospital. Complete recovery.

cholecystitis with or without cholelithiasis and without liver complications were studied. The flocculation was negative in all except 3. The amount of flocculation in each of these 3 patients was slight (+, +, and ++). All had stones in the gall bladder, but it was not possible to demonstrate liver pathology, even though sections from 2 patients were available for microscopic study. Surgery was performed on the gall bladder in 30 of the 34 subjects.

*Flocculation reaction in patients with acute or subacute hepatitis.* Observations were made on 36 patients with hepatitis of an acute or subacute form. There was no evidence of obstruction of the biliary passages in any of these individuals. The diagnosis was established in 12 patients after histological examination of liver tissue. A reduction in plasma prothrombin was observed in 25 of these 36 patients. This observation furnished further evidence that significant liver damage was present (5). Table I presents the diagnosis, the basis for the diagnosis, the cephalin-cholesterol flocculation reaction, icterus index, plasma prothrombin, and the hippuric acid excretion in these patients. The data were collected at the time the diagnosis was established. The cephalin-cholesterol flocculation test was positive in all but 2 of the 36 cases.

Although chronic passive congestion of the liver is not an inflammatory lesion, Cases 26 to 36, inclusive (Table I), are classified with this group

because of the extensive parenchymal destruction. In contrast to these 11 patients with a positive flocculation test, it should be noted that sera from 18 other patients with congestive cardiac failure and similar clinical signs of congestion of the liver, not included in Table I, showed no flocculation.

Repeated observations were made on all of the 36 patients with acute or subacute hepatitis during the course of their disease. A close correlation was usually demonstrated between the amount of flocculation and the clinical severity of the disorder. In each of the individuals who recovered, the positive reaction gradually became weaker, and finally negative as clinical improvement was noted. Table II presents data on a case typical of this group. These observations are in agreement with Hanger's (2) suggestion that the flocculation test has considerable prognostic value.

*Flocculation reaction in patients with cirrhosis of the liver.* Twenty-two patients with various types of cirrhosis of the liver were studied. In most of these individuals the disease was in the terminal stage. Table III gives the diagnosis, the basis for the diagnosis, the cephalin-cholesterol flocculation reaction, icterus index, per cent of plasma prothrombin, and the results of the hippuric acid test. In addition, the serum albumin was reduced, and the globulin abnormally elevated in 17 patients. Table III shows that the flocculation test was uniformly strongly positive in this group of patients. Serial studies showed a

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31	F. W.	M	59	Chronic passive congestion of liver	Autopsy	+++	5	70	0	
32	C. A.	M	39	Chronic passive congestion of liver	Autopsy	+++	75	55		
33	G. B.	M	30	Chronic passive congestion of liver	Clinical	+++	15	45	54	Still under observation.
34	F. D.	F	70	Chronic passive congestion of liver	Clinical	++	3	100		Still under observation.
35	S. W.	F	65	Chronic passive congestion of liver	Clinical	++++	40	10	0	Hemorrhagic diathesis. Died.
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10	++++	50			0 (oral)			Extremely ill. Fever continues. Clinical improvement. Afebrile. Feels well.
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17	+	40		25				
21			1.2		65 (oral)	3.9	2.4	
22	+	20		45				
25	0							
December 2	0	20	less than 0.9	60	78 (intravenous)	4.3	2.1	
6	0	8		100				Discharged from hospital. Complete recovery.
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cholecystitis with or without cholelithiasis and without liver complications were studied. The flocculation was negative in all except 3. The amount of flocculation in each of these 3 patients was slight (+, +, and ++). All had stones in the gall bladder, but it was not possible to demonstrate liver pathology, even though sections from 2 patients were available for microscopic study. Surgery was performed on the gall bladder in 30 of the 34 subjects.

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TABLE III  
Flocculation reaction in 22 patients with cirrhosis of the liver

Case	Initials	Sex	Age	Diagnosis	Basis for diagnosis	Cephalin-cholesterol flocculation	Icterus index	Plasma prothrombin	Hippuric acid test	Comments
			years					per cent	per cent	
1	W. D.	M	49	Atrophic cirrhosis of liver	Clinical	+++++	15	60		Macrocytic anemia. Esophageal varices demonstrated by x-ray. Esophageal varices demonstrated by x-ray. Large nodular liver. Ascites.
2	T. R.	M	63	Atrophic cirrhosis of liver	Clinical	+++++	3	40	33	
3	P. D.	M	39	Atrophic cirrhosis of liver	Clinical	+++++	25	30	0	
4	F. M.	M	41	Atrophic cirrhosis of liver	Clinical	+++++	10	72	47	
5	W. J.	M	60	Atrophic cirrhosis of liver	Autopsy	+++++	20	15	20	
6	A. B.	F	54	Atrophic cirrhosis of liver	Autopsy	+++++	5	100	79	Esophageal varices demonstrated by x-ray.
7	G. M.	M	49	Atrophic cirrhosis of liver	Biopsy of liver	++++	10	55	69	
8	F. C.	M	57	Atrophic cirrhosis of liver. Chronic alcoholism	Clinical	++++	8	65	53	
9	W. S.	M	58	Atrophic cirrhosis of liver. Chronic alcoholism	Clinical	++++	5	100	27	
10	C. A.	M	51	Atrophic cirrhosis of liver. Chronic alcoholism	Clinical	+++++	15	45	43	
11	D. R.	M	37	Atrophic cirrhosis of liver. Chronic alcoholism	Autopsy	+++++	25	45	0	Large firm liver.
12	E. D.	M	59	Atrophic cirrhosis of liver. Chronic alcoholism	Biopsy of liver	+++++	65	70	40	
13	C. M.	M	55	Atrophic cirrhosis of liver. Chronic alcoholism	Clinical	++++	25	100	28	
14	R. T.	M	15	Atrophic cirrhosis of liver. Banti's syndrome	Autopsy	+++++	60	45	16	
15	A. N.	F	52	Obstructive biliary cirrhosis	Operation	++	22	18	15*	
16	C. B.	F	38	Obstructive biliary cirrhosis	Biopsy of liver	++	40	55	31	Cholecystoduodenostomy.
17	F. S.	F	46	Obstructive biliary cirrhosis	Autopsy	+++++	50	45		
18	A. D.	F	60	Obstructive biliary cirrhosis	Biopsy of liver	+++++	60	30	25*	
19	J. P.	F	40	Obstructive biliary cirrhosis	Biopsy of liver	++	40	32	52*	
20	M. H.	F	54	Syphilitic cirrhosis of liver	Clinical	+++++	6	65	54	
21	R. A.	F	42	Syphilitic cirrhosis of liver	Clinical	+++++	45	100	65	Macrocytic anemia.
22	W. C.	M	56	Chronic hepatitis with necrosis	Autopsy	+++++	50	55	7*	

\* Denotes intravenous administration of sodium benzoate.

persistence of the reaction throughout the period of observation. The abnormal hippuric acid test in 18 of these patients, and the prothrombin deficiency in 18, further support the evidence of extensive liver damage. All of the patients with hypoprothrombinemia failed to show a significant increase in this coagulation factor with prolonged administration of crude concentrates of vitamin K or 2-methyl-1, 4-naphthoquinone.<sup>2</sup> The lack of response of the prothrombin to this therapy also indicates an impairment of liver function (5).

*Flocculation reaction in patients with obstructive jaundice.* Observations were made on 23 jaundiced patients who had complete obstruction of the extrahepatic biliary passages. Table IV shows the cause of the obstruction, basis for the diagnosis, approximate duration of the icterus, cephalin-cholesterol flocculation, icterus index, plasma prothrombin, and the hippuric acid test. The data presented in this table were obtained at the time of the patient's admission to the hospital. In addition, the urobilinogen in the urine was less than 0.9 mgm. per 100 cc. in each patient. The flocculation test was positive in 18 of the 23 patients in this group. These results are not in agreement with previous reports (1, 2, 6) which indicate that flocculation does not occur with sera

from patients with biliary obstruction. Comparison of the flocculation reactions of the subjects in Tables I, III, and IV does show, however, that the degree of flocculation was less in the individuals with obstructive jaundice than in those with hepatitis.

The data in Table IV show that the plasma prothrombin was reduced in every patient, and that in a majority the deficiency was marked. Cases 5 and 17 showed hemorrhagic manifestations. The response of the prothrombin to treatment with preparations with vitamin K activity was satisfactory with the exception of Cases 1, 14, 21, and 23. An obstructive biliary cirrhosis or hepatitis was demonstrated histologically in these 4 patients. In addition, a secondary disturbance in the liver was suspected by the surgeon at the time of operation in Cases 5, 19, and 20.

*Flocculation reaction in patients with focal lesions of the liver.* Hanger (2) reported that single or disseminated carcinomatous lesions in the liver were usually accompanied by a negative cephalin-cholesterol flocculation test, although it was recognized that the series was too small to make a definite conclusion. In the present study observations were made on 26 patients with focal lesions of the liver. The group was composed of cases with a variety of lesions which either replaced or invaded liver tissue. Table V presents

<sup>2</sup> Supplied through the courtesy of E. R. Squibb and Sons, New York.

TABLE IV  
Flocculation reaction in 23 patients with obstructive jaundice

Case	Initials	Sex	Age	Diagnosis	Basis for diagnosis	Approximate duration of jaundice	Cephalin-cholesterol flocculation	Icterus index	Plasma prothrombin	Hip-puric acid test	Comments
			years						per cent	per cent	
1	S. S.	F	46		Autopsy	2 weeks	+	50	45	97	
2	W. H.	M	65		Operation	2 months	+++	50	32	69	Post-operative evisceration. Recovered.
3	W. S.	M	57		Clinical	3 weeks	+	30	75	90	Still under observation.
4	E. M.	M	53		Operation	2 months	0	40	75	112	Complete recovery.
5	R. L.	F	44		Operation	10 months	++++	20	35	24	Died of intra-abdominal hemorrhage.
6	B. E.	M	42		Operation	1 week	0	50	80	95	Complete recovery.
7	C. V.	F	50		Operation	3 months	++	35	65	77	Complete recovery.
8	B. F.	M	53		Operation	2 weeks	+++	40	40	84	Complete recovery.
9	L. S.	M	25	Stones in common bile duct	Operation	5 weeks	+	100	32	115	Complete recovery.
10	R. R.	F	41	Stones in common bile duct	Operation	3 weeks	0	60	20	90	Complete recovery.
11	H. G.	M	50	Stone in common bile duct (ball valve)	Operation	2 months	++	25	80		Still under observation.
12	J. P.	M	48	Carcinoma of head of pancreas	Operation	3 months	+++	20	45	82	Still under observation.
13	J. D.	M	62	Carcinoma of head of pancreas	Clinical	1 month	++	75	17	54	Died.
14	F. D.	M	53	Carcinoma of head of pancreas	Autopsy	3 months	0	60	60		
15	W. W.	M	45	Carcinoma of head of pancreas	Operation	3 months	++	60	55	102	Still under observation.
16	S. G.	M	60	Carcinoma of head of pancreas	Operation	6 weeks	0	75	28	86	No follow-up.
17	H. K.	M	55	Carcinoma of head of pancreas	Operation	1 month	++	25	42		Hemorrhagic diathesis.
18	A. S.	M	59	Carcinoma of head of pancreas	Clinical	4 months	++++	80	55	75	Died.
19	C. B.	F	32	Adhesions about common bile duct	Operation	14 months	+++	30	62	60	Cholecystoduodenostomy.
20	M. B.	F	52	Adhesions about common bile duct	Operation	2 months	+	55	35	86	Plastic repair of duct. Recovery.
21	E. B.	F	29	Stricture of common bile duct	Autopsy	18 months	++	30	40	51	
22	E. N.	M	61	Obstruction of common bile duct due to lymph node	Operation	6 weeks	++	100	40	110	Complete recovery.
23	S. D.	F	68	Carcinoma of gall bladder	Autopsy	unknown	+	8	40	83	

TABLE V  
Flocculation reaction in 26 patients with focal lesions of the liver

Case	Initials	Sex	Age	Diagnosis	Basis for diagnosis	Cephalin-cholesterol flocculation	Icterus index	Plasma prothrombin	Comments
			years					per cent	
1	J. C.	M	64	Carcinoma of stomach. Liver metastasis	Autopsy	+++	22	52	
2	R. C.	M	50	Carcinoma of stomach. Liver metastasis	Autopsy	+++	20	32	
3	L. B.	M	70	Carcinoma of stomach. Liver metastasis	Clinical	+++	20	70	Gastric malignancy by x-ray.
4	A. A.	M	54	? Carcinoma of stomach. Liver metastasis	Clinical	+	40	40	Nodular liver.
5	J. L.	M	73	Carcinoma of colon. Liver metastasis	Operation	++++	3	50	Died.
6	H. K.	M	58	Carcinoma of colon. Liver metastasis	Operation	++	3	100	
7	C. M.	M	67	Carcinoma of colon. Liver metastasis	Biopsy of liver	++	20	80	
8	M. L.	F	64	Carcinoma of cervix. Liver metastasis	Clinical	+++	10	22	
9	C. K.	F	11	Carcinoma of breast. Liver metastasis	Autopsy	0	3	60	
10	S. D.	F	68	Carcinoma of gall bladder. Liver metastasis	Autopsy	++	8	40	
11	T. P.	M	78		Operation	+++	75	65	
12	L. M.	F	65		Clinical	++	13	70	Large, nodular liver.
13	R. B.	M	67		Clinical	+++	15	47	Large, nodular liver.
14	A. H.	M	10		Autopsy	++++	10	100	
15	J. H.	M	3	Wilm's tumor. Liver metastasis	Autopsy	++	100	100	
16	L. S.	F	64	Hypernephroma. Liver metastasis	Biopsy of liver	+++	3	60	
17	M. T.	F	65	Leiomyosarcoma of jejunum. Liver metastasis	Autopsy	+	12	100	
18	D. B.	M	12	Lymphosarcoma with liver involvement	Autopsy	+++	10	100	
19	F. T.	M	58	Multiple primary carcinomata of liver	Autopsy	+++	100	60	
20	W. U.	M	38	Multiple abscesses of liver	Operation	++++	5	55	
21	H. S.	M	29	Abscess of liver with biliary fistula	Autopsy	++	75	28	Intra-abdominal hemorrhage.
22	R. B.	M	33	Actinomycosis of liver	Autopsy	+++	4	55	
23	W. M.	M	11	Actinomycosis of liver	Biopsy of liver	++	15	45	
24	M. K.	F	9	Actinomycosis of liver	Autopsy	+++	5	70	
25	M. K.	F	29	Gaucher's disease	Operation	+++	5	65	Splenectomy.
26	R. M.	F	55	Echinococcus cysts of liver	Autopsy	+++	35	100	

the diagnosis, basis for the diagnosis, cephalin-cholesterol test, icterus index, and plasma prothrombin in these subjects. These laboratory results were obtained shortly after admission of the patient to the hospital. Repeated tests were entirely similar. The data show that flocculation occurred in every patient except Case 9. In most instances the reaction was strongly positive. The

plasma prothrombin was reduced in 19 patients in this group, and in cases in which specific therapy was given for this deficiency there was slight, if any, improvement.

#### SUMMARY AND DISCUSSION

The present observations confirm original studies (1, 2) which indicate that emulsions prepared



from mixtures of sheep brain cephalin and cholesterol are flocculated by sera from patients with active disturbances of the liver parenchyma. In the present investigation, sera from 880 human subjects were studied. These data are summarized in Table VI. In this table the patients are grouped in the same manner as they were earlier in this communication when more detailed observations were presented. These results indicate that the incidence of a positive flocculation reaction is very high with sera from individuals with a liver disorder and that false positive reactions rarely occur.

TABLE VI

*Summary of the cephalin-cholesterol flocculation reaction on sera from 880 human subjects*

Diagnosis	Number of patients	Number with negative flocculation reaction	Number with positive flocculation reaction	Per cent of patients with positive flocculation reaction
Normal.....	284	284	0	0
Hospital patients without demonstrable hepatic or biliary tract disease.....	455	440	15	3.3
Cholecystitis without hepatic disease.....	34	31	3	8.8
Acute or subacute hepatitis.....	36	2	34	94.4
Cirrhosis of the liver.....	22	0	22	100.0
Obstructive jaundice.....	23	5	18	78.3
Focal lesions of the liver.....	26	1	25	96.2

The major discrepancy between the present observations and those previously reported (1, 2, 6) resides in the cephalin-cholesterol flocculation reaction with sera from individuals with obstructive jaundice. Hanger (2) concluded that jaundice due to biliary obstruction may be distinguished from hepatogenous jaundice by the flocculation test, since the reaction was usually negative in the former and positive in the latter. In a recent publication (6) Hanger and Gutman distinguished two kinds of postarsphenamine jaundice (hepatitis and intrahepatic biliary obstruction) with the aid of the cephalin-cholesterol flocculation test. These authors noted that flocculation occurred in only 8 of 85 patients (9.4 per cent) with various types of obstructive jaundice. This value is at variance with the present study in which it was noted that flocculation occurred in 18 of 23 such patients (78.3 per cent). The explanation for this lack of agreement is not clear. It is possible that our series of 23 patients with complete biliary obstruction had a higher inci-

dence of secondary disturbances in the liver than Hanger's and Gutman's series, since their group did not all have complete or constant obstruction. It is true in the present study that the test was usually less strongly positive in the patients with obstructive jaundice than in those with a hepatic disorder (Tables I, III, IV, and V). However, the observations herein reported would not permit the use of the cephalin-cholesterol flocculation test in the differential diagnosis of obstructive from hepatogenous jaundice.

There is general agreement that a common disadvantage of the liver function tests which are usually employed is their lack of sensitivity. Often such functional studies remain within normal limits until the end stage of the disorder when a diffuse and irreparable damage has already occurred. Reports by Hanger (2) indicate that the cephalin-cholesterol flocculation test is a more delicate index of active disturbances of the liver than any of the so-called liver function tests. The present investigation confirms this conclusion. The flocculation reaction with few exceptions proved to be a more sensitive indicator of a liver disorder than any of the other tests which were used. This finding is still more impressive when it is recalled that false positive reactions are extremely rare. In several cases an hepatic abnormality, the presence of which was conclusively verified later, was not suspected until the flocculation test was reported positive.

Hanger (1, 2) was unable to demonstrate any correlation between the degree of flocculation of the cephalin-cholesterol complex and the bromsulphalein excretion, hippuric acid formation, levulose tolerance test, Takata-Ara reaction, formol-gel test, the albumin-globulin ratio, serum phosphatase, blood cholesterol or serum bilirubin. He concluded that the flocculation test should be regarded as an index of disturbance of the liver parenchyma and that it did not parallel tests for hepatic function. The present observations do not agree with this statement. Although, as mentioned previously, the flocculation test was usually the most delicate index, our data show that a ++ or stronger reaction was usually accompanied by an abnormal decrease in hippuric acid synthesis and excretion and a reduction in the plasma prothrombin. There was no correlation between these 3 laboratory procedures and the intensity

or duration of jaundice. The serum albumin and globulin were usually abnormal when the liver damage was severe and of long duration.

The cephalin-cholesterol flocculation test, as previously described (2), is of especial value in determining the prognosis of patients with hepatitis. In a majority of individuals with acute or subacute hepatitis, the flocculation reaction decreased or became negative before the icterus receded. The data in Table II illustrate the prognostic significance of the test. We have not observed a recurrence of jaundice in any patient once the test became negative. The persistence of a strongly positive reaction, such as was noted in a few patients with subacute hepatitis and in most of those with cirrhosis or focal lesions of the liver, suggests a poor prognosis despite periods of temporary clinical improvement.

The functions of the liver are multiple and there is no universal test of hepatic function. The tests are modified by many factors such as the type and extent of the liver disease, its acute or chronic nature, the presence or absence of infection, the presence or absence of regeneration of the parenchymal cells and the amount of available protein and glycogen. White, Deutsch, and Maddock (7) have pointed out that certain tests are preferred because of their delicacy, others because of the prognostic value with serial studies. The present data indicate that the cephalin-cholesterol flocculation test is a sensitive and reliable index of a liver disturbance regardless of its etiology, and that it is a valuable adjunct to the other laboratory aids.

#### CONCLUSIONS

1. Emulsions prepared from mixtures of sheep brain cephalin and cholesterol *are not* flocculated by serum from normal individuals and *rarely* by serum from patients without hepatic disease.

2. Cephalin-cholesterol emulsions *are* regularly flocculated by serum from patients with hepatitis, cirrhosis of the liver or focal lesions of the liver.

3. The flocculation test is a more sensitive index of hepatic disease than many of the functional studies. A strongly positive reaction is usually accompanied by a reduction in the plasma prothrombin and hippuric acid synthesis and excretion.

4. The flocculation reaction is usually negative in gall bladder disease without hepatic complications.

5. In the present study the flocculation test was not a reliable guide for the differentiation of obstructive from hepatogenous jaundice.

6. The cephalin-cholesterol flocculation test is a valuable adjunct to other laboratory procedures in the diagnosis and prognosis of jaundiced patients.

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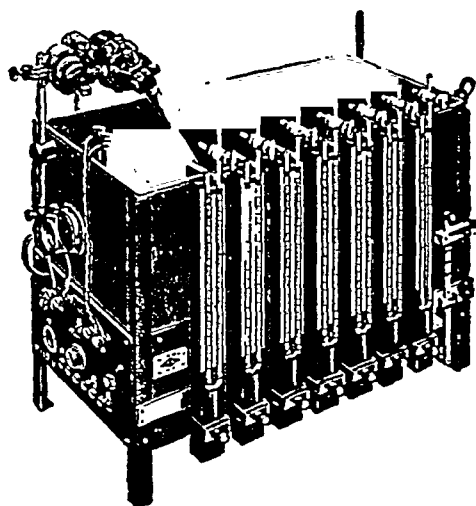
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# FURTHER OBSERVATIONS ON SKIN REACTIONS TO ANTIGENS FROM HETEROLOGOUS CESTODES IN ECHINO- COCCUS DISEASE

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Although the skin test is the most valuable single procedure for the diagnosis of echinococcus disease, its use for either the identification or the exclusion of possible infection is limited because the specific hydatid antigen is not easily obtained in many parts of the world in which suspected cases appear. In recent years, antigens derived from heterologous cestodes have been tried as substitutes for the specific hydatid substance in the immediate type of skin test and, in a number of instances, they have been found to serve the purpose about as well as those from the specific source (1). Several such skin-testing substances have been described previously by the authors (2) who have reported their diagnostic use in a small series of surgically proved cases of echinococcus disease. In the present communication, further observations on skin tests in echinococcus disease are recorded which indicate that many different species of cestodes are a potential source of antigen suitable for eliciting an immediate reaction. Some of these tests were performed directly upon patients known to have the disease, while others were carried out on normal persons by the passive transfer method of Prausnitz and Küstner (3).

## SKIN TESTS IN PERSONS WITH ECHINOCOCCUS DISEASE

### General methods

*The preparation of antigens.* The antigens used for the skin tests were comparatively crude saline extracts of the following helminths: *Echinococcus granulosus* (cyst membranes) from man, *Taenia saginata* from man, *Taenia serrata* from the dog, *Hymenolepis fraterna* from the mouse, *Moniezia expansa* from the sheep, *Raillietina cesticillus* from the chicken, *Dipyllobothrium mansonioides* from the cat, and *Sparganum mansonioides* from the monkey. The isolated cestodes were washed thoroughly with water, dried at 37° C., and triturated in a mortar. Extracts were prepared by suspending 2 per cent of the dried powders in 0.5 per cent phenolated

physiological salt solution for 2 hours at 37° C. The suspensions were then centrifugated and the supernatant fluids decanted from the insoluble residues. Finally, the fluids were proved bacteriologically sterile and preserved in sealed ampules until used.

In addition to these whole-worm extracts, a protein-free polysaccharide derived from *Taenia crassicolis* by Campbell (4) of the University of Chicago was used as a skin-testing antigen. For injection, the dry powder was dissolved in 0.5 per cent phenolated physiological salt solution. The most concentrated solution used contained 10 mgm. of polysaccharide in the 0.1 cc. skin test dose.

*The method of skin testing.* The skin tests were performed on the volar surface of the forearm, 0.1 cc. of each antigenic solution being injected intracutaneously. A positive reaction was considered to consist of a definite wheal, with pseudopodia. This wheal generally appeared within 5 minutes and attained its greatest limits within 10 to 15 minutes. Erythema was commonly noted about the site of injection but was not considered particularly significant. Frequently, when concentrated antigens were injected, delayed reactions characterized by local induration also were noted. These appeared within 30 minutes and sometimes lasted for from 24 to 36 hours.

*The recording of skin reactions.* Immediately after the injection of each antigen, the bleb formed was outlined with ink. The wheal which developed in positive tests was also outlined with ink as soon as its greatest size was attained. Tracings of these outlines were made and are shown in the figures which will be referred to presently.

### Experimental procedure and results

All of the antigens used in this study were tested on a patient who had recently undergone incision and drainage of a large suppurating hydatid cyst of the liver. Many tests with the same antigens were performed at the same time upon a second proved case of echinococcus disease and some were repeated at intervals upon several other known cases of this infection. All of the antigens were also tested on the skins of normal persons.

All of the undiluted extracts elicited vigorous and immediate skin responses in the patients. When these extracts were titrated, however, some tolerated considerably greater dilution than others

before failing to cause a skin reaction. For example, the extract of *Taenia serrata* elicited a reaction even after dilution 2500 times, and that of *Taenia saginata* after 1000 times, whereas the extracts of *Sparganum mansonoides* and *Diphyllbothrium mansonoides* failed to elicit a reaction when diluted more than 50 and 200 times, respectively. The extracts of *Hymenolepis fraterna*, *Moniezia expansa*, and *Railletina cesticillus* could be diluted 500 times and still elicit a skin response. The cyst membranes of *Echinococcus granulosus* evidently contained the smallest available amount of antigen suitable for producing a skin response, since its extract failed to evoke a reaction when diluted more than 10 times. The purified polysaccharide of *Taenia crassicolis* also elicited a reaction in these patients, even when the stock solution, which contained 10 mgm. per 0.1 cc., was diluted 1000 times. Tracings of the immediate reactions obtained after the injection of the various dilutions of all the substances into one of the patients are shown in Figure 1.

None of the antigens caused an immediate reaction in any of the six normal persons tested. In two individuals, the area surrounding the site of injection of the concentrated *Taenia saginata* and *Hymenolepis fraterna* extracts promptly became slightly edematous, but the margins of the bleb never extended significantly, and no pseudopodia were formed. When these antigens were diluted as little as 5 times, no reaction whatsoever was elicited in any individual. Occasionally, in some of the normal persons, an erythematous zone about an inch in diameter developed at the site of injection 3 or 4 days after the inoculation of the antigens, but this disappeared within the next 24 hours or so, and seemed to have little or no relationship with the immediate positive reaction observed in the patients.

#### SKIN TESTS IN PERSONS SENSITIZED LOCALLY WITH SERUM FROM A PATIENT WITH ECHINOCOCCUS DISEASE (PRAUSNITZ-KÜSTNER TESTS)

##### *General methods*

Serums from two patients with echinococcus disease and from a normal person were diluted with two parts of physiological salt solution and passed through a sterile Seitz E-K filter. One-tenth cubic centimeter of each filtrate was then inoculated intracutaneously into a series of well-separated sites in the upper arm of two normal

individuals. Forty-eight hours later, these prepared sites, as well as the control sites, were injected with the stock antigenic extracts of *Taenia serrata*, *Taenia saginata*, *Hymenolepis fraterna*, *Moniezia expansa*, *Railletina cesticillus*, and *Diphyllbothrium mansonoides*, and with a sample of hydatid cyst fluid. The reactions were read and recorded in the same manner as the direct tests upon the infected patients. The results of these tests with the serum from one patient and appropriate controls, as performed on two normal persons, are presented in Figure 2.

#### RESULTS

In all of the sites prepared by injection with the filtrates of the serums from the patients, a definite reaction to all the cestode antigens occurred, with wheals showing pseudopodia and attaining their greatest size in about 10 minutes. Delayed reactions, although in some cases definite, were mild when compared with those following the direct tests in the patients with the hydatid infection. No reaction whatsoever was observed at any of the sites prepared with the filtrate of normal serum, or at any of the antigen control sites.

#### DISCUSSION

The foregoing experimental work indicates that antigens capable of eliciting a skin reaction in hydatid patients can be derived from many different species of cestodes. Suitable antigens seem to occur in greatest amount in cestodes closely related to the homologous parasite, and proportionately less in distantly related forms. It is noteworthy that the homologous hydatid membrane is particularly poor in the skin-testing substance.

The presence of similar antigens in related cestodes agrees in principle with what is known for other classes of helminths. For example, antigens from apparently any mammalian schistosomum serve in skin tests for human schistosomiasis (5). Suitable antigens for this purpose are said also to occur in the comparatively remotely related liver flukes (6). Likewise, among nematodes, a considerable community of antigens is apparent. The dog heartworm *Dirofilaria immitis*, for example, yields antigens useful for skin testing in infections with several different human filarias (7), and a rabbit oxyurid, *Passalurus ambiguus*, has provided antigens for identifying infection with the human pin-worm, *Enterobius vermicularis* (8). The most inclusive group-reaction

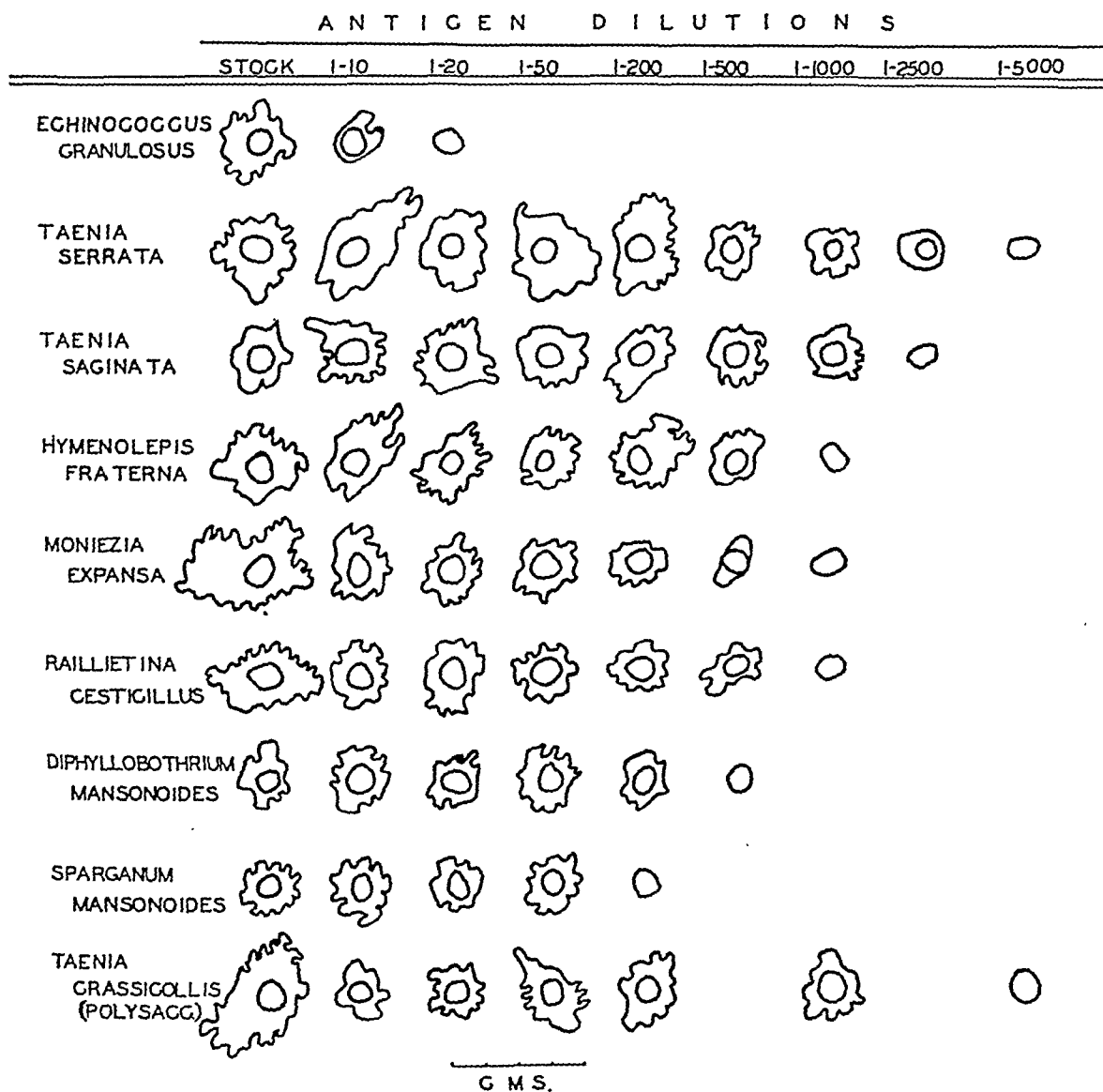


FIG. 1. SKIN REACTIONS IN A PATIENT WITH ECHINOCOCCUS DISEASE AFTER INJECTING PROGRESSIVE DILUTIONS OF EXTRACTS OF THE FOLLOWING CESTODES: *Echinococcus granulosus* (HYDATID MEMBRANES) FROM MAN, *Taenia serrata* FROM THE DOG, *Taenia saginata* FROM MAN, *Hymenolepis fraterna* FROM THE MOUSE, *Moniezia expansa* FROM THE SHEEP, *Railletina cesticillus* FROM THE CHICKEN, *Diphyllobothrium mansonoides* FROM THE CAT, AND *Sparganum mansonoides* FROM THE *Macacus rhesus*

Tests with a protein-free polysaccharide derived from cysticerci of *Taenia crassicollis* from the rat are also shown.

The inner circle represents the outline of the initial bleb, and the outer irregular line the greatest limits attained by the wheal in positive reactions.

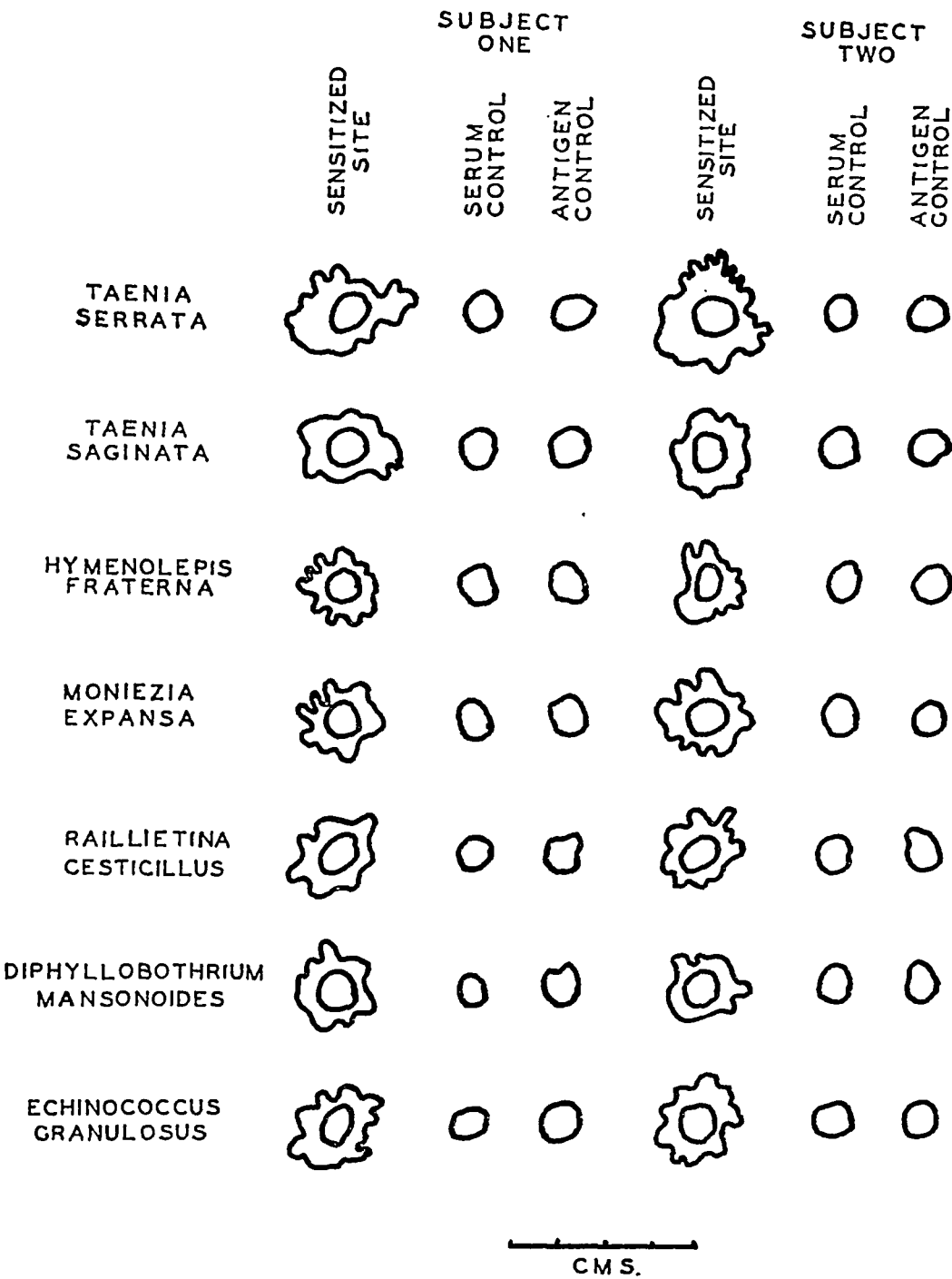


FIG. 2. PRAUSNITZ-KÜSTNER TESTS IN TWO NORMAL SUBJECTS WITH ANTIGENS DERIVED FROM *Taenia serrata*, *Taenia saginata*, *Hymenolepis fraterna*, *Moniezia expansa*, *Raillietina cesticillus*, *Diphyllobothrium mansonoides*, and *Echinococcus granulosus*

Tests were carried out with each antigen in sites sensitized with serum from a patient with hydatid disease, or with normal serum, and in previously unprepared sites. The small circle represents the limits of the initial bleb after the antigen injection. The irregular outer line represents the greatest limits of the wheal in positive tests.

among the nematodiasis has been reported by Brunner (9), who concluded that an extract of *Ascaris lumbricoides* will elicit a skin test in persons with any nematode infection.

Because of the group character of the reaction in the echinococcus skin test, positive responses obtained with antigens from any of the cestodes mentioned should be viewed critically before acceptance as evidence for infection with echinococcus disease. The possibility of infection with some other type of *somatic* cestode (e.g., cysticercosis, sparganosis) should especially be borne in mind because powerful and confusing skin responses can be expected from them.<sup>1</sup> Most such infections, however, can be differentiated from echinococcus disease with little difficulty through clinical procedures. Furthermore, the intestinal cestode infections also must be ruled out because they sometimes produce skin reactions. The exclusion of these intestinal infections can generally be accomplished rather simply through careful stool examinations. It is therefore the opinion and experience of the authors that, in persons suspected of hydatid infection on clinical grounds after other cestode infections have been excluded, a positive skin test with any of the antigens described provides critical evidence for the presence of this disease, especially when the test is performed with a well-diluted antigen.

Considering the observations presented here from a purely practical standpoint, passive transfer reactions to antigens prepared from cestodes such as *Taenia serrata* may offer an additional method of diagnosis in echinococcus disease which has an advantage in certain extraordinary circumstances. For instance, specimens of serum obtained for the Ghedini-Weinberg complement-fixation reaction (11) from patients residing at a distance may also be used to perform Prausnitz-

Küstner tests, thus skin-testing the patient, so to speak, by proxy.

#### CONCLUSION

Antigens suitable for eliciting skin reactions in patients with echinococcus disease can be derived from many different cestodes, including (in addition to the specific larval parasite *Echinococcus granulosus*) *Taenia serrata*, *T. saginata*, *T. crassicolis*, *Hymenolepis fraterna*, *Moniezia expansa*, *Raillietina cesticillus*, and both the adult and sparganum of *Diphyllobothrium mansonoides*.

These antigens will also elicit skin reactions in normal persons locally sensitized passively with the serum from patients with echinococcus disease.

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<sup>1</sup> Two human cases of experimentally induced sparganosis (*Diphyllobothrium mansonoides*) have been skin tested by Mueller (10) of the New York State College of Forestry with antigenic extracts supplied by the authors. Positive tests were obtained with extracts of *Diphyllobothrium mansonoides* and *Taenia serrata*, prepared as described in this paper, as well as with the cyst fluid of *Echinococcus granulosus*. The reactions were of the immediate type with definite pseudopodia and erythema, while local induration persisted for some hours thereafter.



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# CALCIUM AND PHOSPHORUS METABOLISM IN OSTEOMALACIA.

## XI. THE PATHOGENETIC RÔLE OF PREGNANCY AND RELATIVE IMPORTANCE OF CALCIUM AND VITAMIN D SUPPLY

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Though osteomalacia is fundamentally a disease due to vitamin D deficiency and dietary calcium shortage, it is made manifest or aggravated by a host of predisposing factors among which the processes of reproduction deserve special attention. Pregnancy and lactation, albeit normal physiological phenomena, make additional demands upon the calcium and phosphorus supplies of the mother, so that diets adequate under ordinary circumstances become deficient during reproductive activity. Our previous reports (1, 2) on the metabolic data of eight Chinese lactating women, with or without osteomalacia, have demonstrated that the drain of lactation varies with the stage of lactation and the quantity of milk secretion. During late lactation when milk yield is small, positive balances in calcium and phosphorus can usually be secured with the addition of vitamin D, even when the intake of minerals is moderate or limited. However, in early lactation, especially if abundant, the loss of calcium in milk is so great that a high intake of calcium is essential in addition to adequate vitamin D supply to prevent depletion of skeletal store. Therefore, if nursing is maintained on dietaries deficient in vitamin D and calcium, as is often the case in China, skeletal demineralization will inevitably result.

A similar chain of events probably occurs in pregnancy. In this part of the reproductive cycle nutritional requirements are increased to provide building material for the fetus and its adnexa and for the development of maternal tissues such as the uterus, mammary glands and other organs, in order to meet the demands of labor and parturition and to prepare for milk secretion. The quantitative aspects of the question are not accurately known, but may be approached from the chemical analysis of fetuses at term and at various ages. Givens and Macy (3) and Macy and Hunscher (4) have shown the average calcium content of

the human fetus at birth to be about 21 to 23 grams; Coons *et al.* (5) give similar estimates, while McIlroy (6) puts the figure considerably higher, namely, at 30 grams. The average phosphorus content of the fetus at term is approximately 14 grams. The whole subject has been reviewed by Macy and Hunscher (4) and by Garry and Stiven (7). It is generally accepted that the mineral needs of the fetus are insignificant during the first four months, but from then on they increase rapidly so that about two-thirds of the total are deposited during the last three months. Therefore, a minimum of 200 mgm. of calcium and 100 mgm. of phosphorus per day should be retained by the mother during the last three months of pregnancy in order to satisfy the fetal requirement without drawing upon the maternal mineral store. These estimates, though they are from Western sources, may serve to indicate the magnitude of drain upon the maternal tissues during pregnancy if living conditions and dietaries are incapable of supporting such a degree of mineral retention, as they appear to be frequently in China.

The primary purposes of the work to be reported in the present communication are to observe the calcium, phosphorus and nitrogen metabolism of patients with osteomalacia during pregnancy, to compare it with that of individuals without skeletal decalcification and to assess the relative importance of vitamin D, calcium and phosphorus intake in securing adequate mineral balances for the added requirements of gestation.

### PROCEDURE

All the patients were studied in the metabolism ward where diets were quantitatively prepared and served, and excreta completely collected. The diets were practically free from vitamin D except those containing small amounts of eggs. They were low in calcium but, when desired, the calcium intake was raised by administering

TABLE I  
*Composition of diets in grams per day*  
(Values for calcium, phosphorus and nitrogen are actually analyzed.)

Case.....	1				2				3				4				5				6				7				8				9		10																																																																																																																																																																																									
Periods (4-day)	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1-2 3-4 5-6 7-8 9-10 11-13	1

a 7.7 per cent solution of calcium lactate. Distilled water was used for cooking and drinking. The diets were quantitatively consumed with the exception of a few instances. The refused food or vomitus, as the case may be, was then separately analyzed and subtracted from the day's intake. The ward routines and chemical methods for the analysis of food, excreta and serum were described previously (1, 2, 8). The metabolic periods were four days each.

RESULTS

This study includes ten Chinese women admitted during various stages of pregnancy. For convenience in presentation, these subjects may be divided into 3 groups according to the condition of their skeletal system. Group I consists of three cases which may be considered, for our purpose, as normal controls, there being no tetany, nor roentgenologic evidence of osteomalacia. Group II contains four subjects, all of whom showed mild osteoporosis, and three of whom had active tetany prior to the metabolic studies. Group III is composed of three patients with advanced osteomalacia, with marked skeletal rarefaction, deformity and fractures. This classification is only approximate because slight depletion of the mineral contents of bones may be passed as normal and small differences in the density of bones from case to case are not detectable by x-ray examination. Moreover, the current state of vitamin D nutrition, as shown by the metabolic behavior at the moment, may not always correspond with the condition of the skeleton. However, there is a general parallelism between the skeletal condition and the state of vitamin D store as revealed by metabolic observation in those patients receiving no prior vitamin D medication.

Group I. Normal

Case 1, K. C. H. This was a 19-year-old primipara with normal serum calcium and inorganic phosphorus and without clinical or roentgenologic evidence of skeletal decalcification. She was observed for 13 four-day periods from the end of the eighth month of gestation to term. Two diets, one low in both calcium and phosphorus and the other low in calcium but moderate in phosphorus, alternated with each other every 2 periods. As seen from Figure 1 and Table II, an intake of 141 to 150 mgm. calcium (Periods 1 to 4) gave

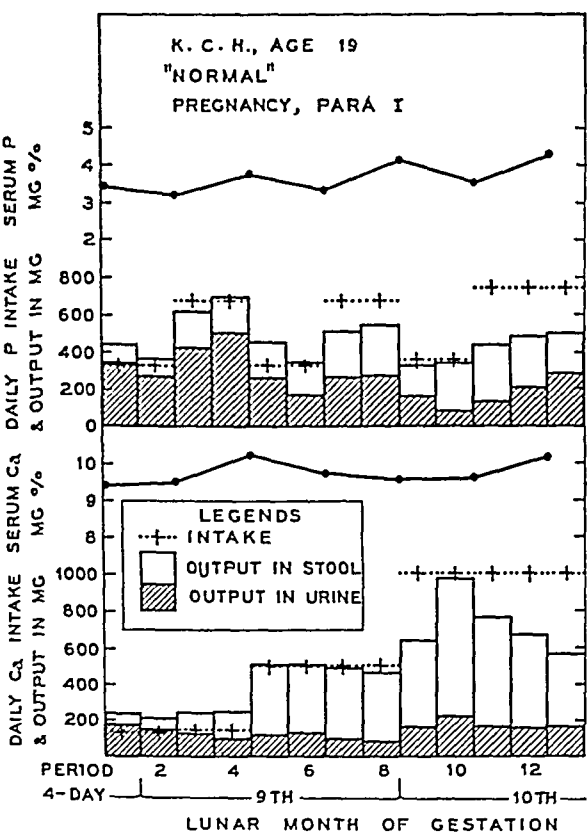


FIG. 1. CASE 1. CALCIUM AND PHOSPHORUS METABOLISM IN A PRESUMABLY NORMAL INDIVIDUAL DURING THE LATTER PART OF PREGNANCY

rise to considerable negative balance, averaging 92 mgm. per day. Raising the intake to 500 mgm. (Periods 5 to 8) elicited barely even balances. On further augmenting the intake to 1000 mgm. (Periods 9 to 13), one notices a considerable calcium gain, namely, 284 mgm. per day. The calcium balance at a given level of calcium intake was not influenced by the different amounts of phosphorus in the two diets.

However, phosphorus balances were naturally dependent on the phosphorus intake. With an intake of 325 to 358 mgm. per day (Periods 1 to 2, 5 to 6 and 9 to 10), the balances were in the main negative, averaging 44 mgm. per day; while an intake of 674 to 741 mgm. (Periods 3 to 4, 7 to 8 and 11 to 13) gave rise to considerable gain, namely, about 160 mgm. per day. The extent of phosphorus retention at a given level of intake also depended upon the calcium intake. As the latter was gradually raised from 150 to 1000 mgm., there was a progressive diminution of the

negative phosphorus balance on the low phosphorus intake and a similar increase of the positive phosphorus balance on the moderate phosphorus intake. This dependence of both calcium and phosphorus balances upon the calcium intake indicates that calcium is a more important limiting factor in the metabolism of the two elements, an observation which has already been described in our previous studies (9, 10).

Nitrogen balances remained fairly satisfactory. Serum calcium did not vary significantly throughout the periods of observation, but there was a tendency for serum inorganic phosphorus to fluctuate with the phosphorus intake.

In a previous communication (11), it has been shown that the earliest sign of vitamin D deficiency is a diminution or disappearance of urinary calcium. Increase of stool calcium, decrease of calcium balance and changes in serum calcium and phosphorus follow in that order as the deficiency is allowed to go on. This patient was able to maintain good amounts of calcium in the urine throughout the 13 periods of study. This, together with the consistently normal serum calcium and phosphorus, indicates that the patient had an adequate store of vitamin D during the studies. Most of that store probably had been acquired prior to admission, as only one of the diets served on the ward contained any vitamin D-containing food, namely egg, and that in small amounts only.

With vitamin D operative, one may perhaps expect this patient to keep definitely positive balances on an intake of 500 mgm. calcium a day. However, only even balances were obtained, and this suggests that her usual intake had been at that level because, as previously pointed out (1), the calcium requirement depends upon, among other factors, the customary dietary level. If 500 mgm. be the usual level of intake, considerably more should be supplied during pregnancy to meet the fetal needs. In this patient an intake of 1000 mgm. of calcium enabled her to retain a sufficient amount for the added requirement.

*Case 2, W. T.* Though this 17-year-old primipara had a normal skeleton, her serum calcium and phosphorus were somewhat lower than normal. Metabolic studies extended for 17 periods from the sixth to the eighth lunar month of gestation (Table II). This subject was given 3 diets, all low in calcium, but progressively increased in

TABLE II  
Group 1. Normal calcium, phosphorus and nitrogen metabolism

Case	Date	Period 4-day	Stage of gesta- tion	Calcium, average daily				Phosphorus, average daily				Nitrogen, average daily			
				Intake	Urine	Stool	Balance	Intake	Urine	Stool	Balance	Intake	Urine	Stool	Balance
			<i>lunar months</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>
1. K.C.H.	1937														
	March 29-April 5	1-2	8-9	141	164	60	- 83	325	306	96	- 77	7.68	6.74	0.62	+0.32
	April 6-13	3-4	9	150	116	134	-100	674	459	196	+ 19	8.92	7.88	0.65	+0.39
	14-21	5-6	9	500	128	376	- 4	325	216	182	- 73	7.68	5.92	0.70	+1.06
	22-29	7-8	9	500	97	385	+ 18	674	268	259	+147	8.92	6.52	0.64	+1.76
	May 30-May 7	9-10	10	1000	200	612	+188	358	122	218	+ 18	8.45	6.68	0.64	+1.13
	8-19	11-13	10	1015	165	502	+348	741	208	266	+267	9.81	7.68	0.65	+1.48
2. W.T.	1936-37														
	December 27-January 3	1-2	6	189	39	256	-106	341	327	240	-226	8.50	8.40	1.01	-0.91
	January 4-11	3-4	6-7	192	29	162	+ 1	565	445	171	- 51	7.84	7.29	0.74	-0.19
	12-19	5-6	7	268	20	220	+ 28	826	491	258	+ 77	9.98	8.57	0.87	+0.54
	20-27	7-8	7	500	57	380	+ 63	426	206	295	- 75	10.63	7.80	1.13	+1.70
	28-February 4	9-10	7	500	9	414	+ 77	706	363	247	+ 96	9.80	7.20	0.86	+1.74
	February 5-12	11-12	8	500	4	444	+ 52	1032	458	388	+186	12.48	8.21	1.16	+3.11
	13-20	13-14	8	1000	20	682	+298	426	378	350	-302	10.63	7.94	1.26	+1.43
	21-28	15-16	8	1000	4	809	+187	706	288	324	+ 94	9.80	8.04	0.94	+0.82
	March 1- 4	17	8	1000	12	698	+290	1032	329	333	+370	12.48	9.31	1.07	+2.10
3. L.C.P.	1940														
	March 11-22	1-3	7	277	3	138	+136	1296	544	463	+289	14.67	9.22	1.99	+3.46
	23-April 3	4-6	8	1314	6	939	+369	837	426	294	+117	12.34	9.66	1.15	+1.53
	April 4-15	7-9	8	277	5	507	-235	1296	581	566	+149	14.67	9.15	2.29	+3.23
	16-27	10-12	8-9	1314	7	820	+487	837	437	223	+177	12.34	8.54	0.99	+3.45
	28-May 9	13-15*	9	277	2	326	- 51	1296	614	500	+182	14.67	8.89	1.74	+4.04
	May 10-21	16-18	9	1314	297	475	+542	837	281	242	+314	12.34	8.54	1.28	+2.52
	22-June 2	19-21	10	218	21	128	+ 69	1277	552	504	+221	14.48	8.44	2.05	+3.99
	June 3-14	22-24	10	1257	118	446	+693	708	189	261	+258	12.65	7.91	1.06	+3.68

\* Vigantol during Periods 13-16.

phosphorus content. She showed similar metabolic behavior to Case 1 in that negative calcium balances (—26 mgm. daily) prevailed on an intake of 189 to 268 mgm., slightly positive balances (64 mgm. daily) were obtained on an intake of 500 mgm. and substantial gain (253 mgm. daily) was secured on an intake of 1000 mgm. This is also true with phosphorus balances which varied directly not only with the level of phosphorus intake but also with that of calcium intake.

In comparison with the first case, although this individual exhibited the same degree of conservatism in handling calcium and phosphorus, her store of vitamin D was probably not as adequate, in that the levels of serum calcium and phosphorus were not strictly normal and urinary calcium tended to decrease and disappear even on high intake. Although small amounts of eggs were present in the diet, they were evidently not sufficient to prevent a gradual depletion of the scanty vitamin D store of the body. However, an intake of 1000 mgm. of calcium seemed adequate to fulfill the requirements of pregnancy even if the vitamin D store was somewhat inadequate.

Case 3, Mrs. L. C. P. This pregnant woman, para IV, was considered normal from the standpoint of her skeleton, and her serum calcium and

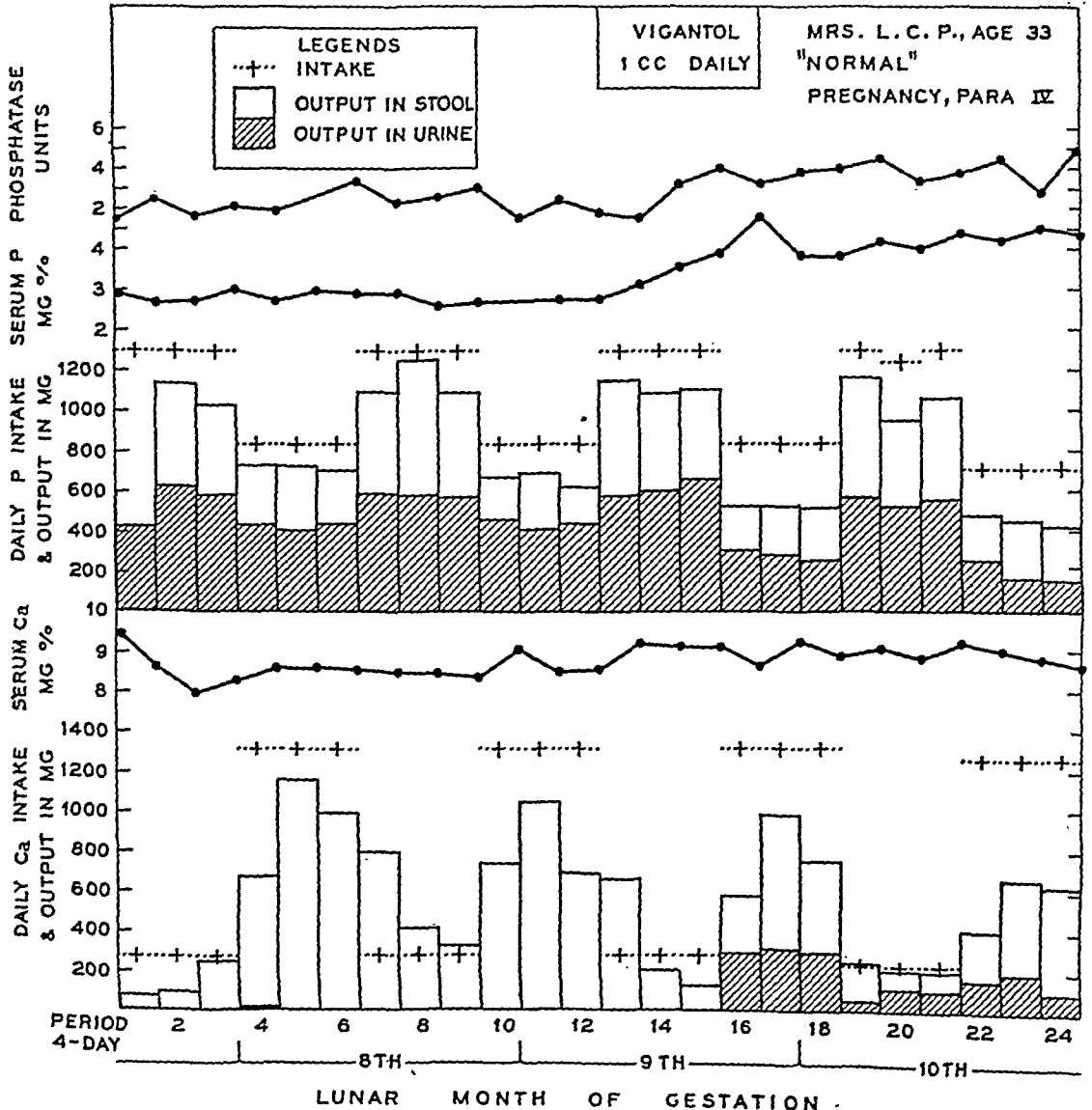
phosphorus on admission were essentially normal. The plan of observation in this case consisted of 3 periods of low calcium-high phosphorus intake followed by 3 periods of high calcium-moderate phosphorus intake; and this cycle was repeated 3 more times. Therefore, the study covered 24 periods from the seventh month to term. The data are set forth in Figure 2 and Table II. During the first 3 periods on low calcium diet (277 mgm.), the patient exhibited extraordinary ability in maintaining positive balance, averaging 136 mgm. per day. During the next 3 periods on high calcium intake (1296 mgm.), the average positive balance of 369 mgm. per day was likewise satisfactory. When the cycle of dietary regime was repeated, though the calcium balance was excellent on high intake (Periods 10 to 12), it was markedly negative on low intake (Periods 7 to 9), indicating poor conservation. This, together with a tendency for the serum calcium to fall during the low calcium periods, suggests the wearing out of whatever vitamin D store the patient might have had at the beginning of the studies.

The supply of vitamin D as Vigantol 1 cc. per day (12,000 international units) for Periods 13 to 16 brought about striking changes. From Pe-

riod 13 to 15, the first 3 periods of Vigantol administration, when the effect of the therapy could hardly be maximal, the calcium balance on low intake began to show favorable influence. In the next 3 periods (Periods 16 to 18), while on high calcium intake, the stool calcium was much reduced, and the urinary calcium much increased. As the reduction of calcium in the stool was greater than the increase of urinary calcium, the

net balances exceeded those of previous periods on similar high intake. The last 6 periods showed even better calcium retention. During Periods 22 to 24, the average daily retention was 693 mgm., namely, 55 per cent of the intake.

Phosphorus balances remained positive throughout, more so after Vigantol administration. Periods of high phosphorus intake were not necessarily associated with greater phosphorus retention



than periods of moderate phosphorus intake. In fact, the average daily retention during all the moderate phosphorus periods was slightly more than that during all the high phosphorus periods, undoubtedly because of the limiting effect of low calcium intake during the latter.

Serum calcium and phosphorus both showed a tendency to rise after vitamin D therapy. The rise of serum phosphorus was particularly striking. The nitrogen balance remained excellent throughout.

In comparison with the preceding case, this patient probably had even scantier vitamin D store, because the urinary calcium was absent from the beginning of the experiment. The unusual ability to maintain a positive calcium balance on a low intake during the first 3 periods is to be explained by her previous low calcium intake, which was quite likely the case. In agreement with this supposition is her inability to maintain a balance when the low calcium intake periods were repeated immediately after 3 periods of high intake. However, further depletion of the scanty vitamin D store probably played a contributory rôle in the difference in behavior between the first and second series of low calcium intake periods.

In spite of inadequate vitamin D store, an intake of approximately 1300 mgm. of calcium resulted in adequate retention for the heightened requirements of pregnancy. However, the supply of vitamin D constitutes a more fundamental solution to the problem. Thus, after Vigantol administration in this case, not only did the urinary calcium appear, the calcium balances on high intake improve and the serum calcium and phosphorus rise to normal, but also positive balances were maintained on low intake. With adequate vitamin D supply the calcium intake necessary for the requirements of pregnancy could be considerably reduced from 1.3 grams.

*Comment.* These three patients are alike in possessing normal skeletal mineral store, but they differ in the state of vitamin D nutrition. The first subject apparently had an adequate store of vitamin D so that 13 four-day periods of a diet low in vitamin D failed to elicit any evidence of depletion. The average daily calcium retention on high intake was 284 mgm., namely 28 per cent of the intake. The vitamin D store of the second subject was not so adequate, in that signs of de-

pletion began to occur after a similar period of study. However, the extent of calcium retention was approximately the same, namely 263 mgm. on high intake, or 26 per cent. The third patient showed evidence of depletion even earlier in the course of observation than in Case 2. Still, the extent of calcium retention on high intake remained satisfactory (28 per cent during Periods 4 to 6 and 37 per cent during Periods 10 to 12). These observations indicate the frequency of the existence of early or subclinical vitamin D deficiency as in the second and third patients. Such deficiencies cannot be recognized unless detailed metabolic observations are made. In such cases, however, high calcium intake (1 to 1.3 grams) exerts an ameliorative influence and even promotes sufficient calcium retention for the augmented requirements of gestation. On the other hand, vitamin D is such an economizer of calcium that in the presence of a lower level of calcium, as is the rule here, an adequate supply of vitamin D is imperative, especially during periods of reproductive activity.

#### *Group II. Early or mild osteomalacia*

*Case 4, Mrs. L. C. F.* Though this 19-year-old primipara had no history or clinical evidence of osteomalacia or tetany, a roentgenologic survey of the skeleton showed slight but definite osteoporosis. Similar to Case 3, a low calcium-high phosphorus regimen alternated with a high calcium-moderate phosphorus regimen, covering a total of 18 four-day periods from the eighth month of gestation to term. The first series of 6 periods (Table III) witnessed a slightly positive calcium balance on an intake of 215 mgm. per day and a substantial gain (averaging 401 mgm. daily or 31 per cent) on an intake of 1275 mgm. per day. But, as the studies proceeded, a negative balance prevailed on a low intake, and retention on a high intake steadily diminished so that during the last 2 periods hardly any calcium was retained. This extraordinary behavior indicates the markedly defective intestinal absorption of calcium usually seen in advanced vitamin D deficiency.

Phosphorus balances were slightly positive throughout, but they tended to be less so with progress of time, corresponding to the behavior of calcium balances. Serum calcium remained constantly between 8 and 9 mgm., while inorganic

phosphorus, slightly above 3 mgm. at the beginning, went down to 2 mgm. per cent towards the latter part of the studies. Phosphatase was slightly above normal, mostly between 4 and 6, but on occasions above 7 Bodansky units.

The point worthy of note in this patient is that, in severe vitamin D depletion, even an intake as high as 1275 mgm. calcium may not enable the patient to maintain a positive balance. This fact may serve to support the contention that adequate vitamin D plays a more important rôle than high calcium intake in promoting calcium gain.

*Case 5, Mrs. S. P. S.* This subject, aged 29 years, para IV, may be characterized as a case of mild osteomalacia and latent tetany. Her studies during 7 four-day periods between the fifth and sixth months of gestation showed slightly negative calcium balances on an intake of 260 mgm. and an average daily retention of 363 mgm. on an intake of 1197 to 1266 mgm. (Table III). The same dietary regimen was repeated eleven days after spontaneous abortion of twin fetuses. Both the negative balances in calcium on low intake and the positive balances on high intake (averaging 302 mgm. daily) were essentially the same as those during pregnancy. Nor were there pronounced differences in phosphorus retention between the observations during pregnancy and those after delivery. However, there was an unquestionable tendency for both serum calcium and phosphorus to rise after parturition. Whereas before delivery serum calcium varied between 7.14 and 8.47 mgm., its range after delivery was between 7.24 and 8.96 mgm. per cent. Likewise, serum phosphorus, varying between 1.73 and 2.24 during pregnancy, was from 2.11 to 3.47 mgm. per cent after delivery.

In this patient moderate vitamin D deficiency was present, as evidenced by the low serum calcium and phosphorus, the absence of calcium in urine and the failure to retain larger amounts of calcium than she did in the face of mineral shortage in the skeleton.

This patient gave us the opportunity to compare the metabolic behavior of the same individual during pregnancy with her behavior postpartum and uncomplicated by lactation. The results revealed no essential difference between pregnancy at the fifth and sixth months and reproductive rest, as far as the mineral balances were concerned.

Of course, one is aware of the fact that during pregnancy a goodly portion of the retained mineral goes to supply the fetus and its adnexa, while during reproductive rest all remains in the maternal tissues. This is probably the explanation for the tendency of the serum calcium and phosphorus to rise after the termination of the pregnancy without any change in the dietary regimen and without any addition of vitamin D, as shown by this patient.

However, there was no extra demand over and above what was required by the products of conception for growth and development. This is in distinct contrast to the state of affairs in active and early lactation where, it has been demonstrated (1, 2), the metabolic processes are so greatly stimulated that calcium has to be supplied not only to cover what is secreted in the milk, but also to cope with this less well-defined factor of stimulation. This is true even in the presence of adequate vitamin D supply. From this we may infer that the drain of pregnancy, as a rule, is not as great as that of lactation.

*Case 6, Mrs. C. W. C.* This woman of 31 years of age, with mild osteomalacia, cataract and tetany for many years, was studied during the eighth to tenth months of her fourth pregnancy. Prior to the commencement of metabolic observations, calcium gluconate and small amounts of vitamin D were given so that tetany was controlled. Metabolic behavior for the first 3 periods on a daily intake of 212 mgm. of calcium (Table III) was conservative in that positive balances were observed. With the intake raised to 1257 mgm. per day, the daily calcium retention averaged 416 mgm. (Periods 4 to 6). When the low calcium diet was repeated during Periods 7 to 9 and 13 to 15, negative balances prevailed, partly because of the preceding high calcium regimen and partly because of beginning depletion of the scanty store of vitamin D acquired prior to the studies. However, with high calcium intake during Periods 10 to 12 and 16 to 18, the patient had no difficulty in securing adequate positive balances which were, respectively, 324 and 474 mgm. per day.

Phosphorus balances were, on the whole, positive, the extent of retention varying with the level of phosphorus intake as well as with that of calcium intake. Serum calcium fluctuated irregu-



TABLE III

*Group 2. Early or mild osteomalacia. Calcium, phosphorus and nitrogen metabolism*

Case	Date	Period 4-day	Stage of gestation	Calcium, average daily				Phosphorus, average daily				Nitrogen, average daily			
				Intake	Urine	Stool	Balance	Intake	Urine	Stool	Balance	Intake	Urine	Stool	Balance
			<i>lunar months</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>
4 L.C.F.	1939-40														
	October 30-November 10	1-3	8	215	6	172	+ 37	926	369	426	+131	9.69	5.64	2.21	+1.84
	November 11-22	4-6	8-9	1275	5	868	+402	692	282	249	+161	8.69	5.63	1.58	+1.48
	December 23-December 4	7-9	9	215	5	319	-109	926	505	419	+ 2	9.69	6.54	1.89	+1.26
	December 5-20	10-13	9-10	1275	4	1066	+205	692	288	308	+ 96	8.69	4.65	1.82	+2.22
	January 21-January 1	14-16	10	215	4	301	- 90	926	435	377	+114	9.69	5.14	1.59	+2.96
	January 2- 9	17-18	10	1275	4	1252	+ 19	692	267	364	+ 61	8.69	4.57	1.67	+2.45
5 S.P.S.	1940														
	February 7-18	1-3	5	260	0	314	- 54	1199	500	576	+123	12.59	7.88	2.06	+2.65
	February 19-26	4-5	5-6	1197	2	786	+409	782	324	436	+ 22	12.10	8.10	1.76	+2.24
	March 27-March 5*	6-7	6	1266	2	948	+316	753	238	374	+141	11.77	7.92	1.87	+1.98
	March 18-29	11-13		260	2	322	- 64	1199	660	494	+ 45	12.59	9.23	1.52	+1.84
	March 30-April 10	14-16		1266	4	960	+302	753	256	278	+219	11.77	7.58	1.27	+2.92
6 C.W.C.	1939-40														
	November 19-30	1-3	8	212	9	145	+ 58	1075	588	349	+138	14.19	7.8	1.93	+2.48
	December 1-12	4-6	8	1257	21	821	+415	703	399	234	+ 70	9.42	7.29	1.37	+0.76
	December 13-24	7-9	8-9	212	2	342	-132	1075	458	413	+204	14.19	8.23	1.56	+4.40
	January 25-January 5	10-12	9	1257	2	931	+324	703	281	244	+178	9.42	5.90	1.25	+2.27
	January 6-17	13-15	9-10	222	5	398	-181	1123	509	452	+162	15.06	9.17	1.76	+4.13
	January 18-29	16-18	10	1257	3	780	+474	703	316	283	+104	9.42	7.12	1.19	+1.11
7 W.E.T.	1937														
	March 13-20	1-2	5	160	48	163	- 51	1026	402	470	+154	10.49	7.95	1.53	+1.01
	March 21-28	3-4	5	205	41	82	+ 82	651	388	328	- 65	9.30	6.93	1.27	+1.10
	April 29-April 5	5-6	5-6	142	48	62	+ 32	308	141	176	- 9	8.64	6.68	1.28	+0.68
	April 6-13	7-8	6	1000	70	640	+290	1026	128	630	+268	10.49	7.52	1.56	+1.41
	April 14-21	9-10	6	1000	44	872	+ 84	651	274	447	- 70	9.30	7.84	1.20	+0.26
	April 22-29	11-12	6	1000	74	710	+216	308	84	242	- 18	8.64	6.85	0.95	+0.94
	May 30-May 7	13-14†	7	160	18	256	-114	1026	280	564	+182	10.49	7.05	1.46	+1.98
	May 8-15	15-16†	7	205	33	60	+112	651	326	219	+106	9.30	6.34	1.34	+1.62
	May 16-23	17-18	7	142	58	46	+ 38	308	136	140	+ 32	8.64	5.87	1.31	+1.46
	May 24-31	19-20	7-8	1000	188	438	+374	1026	136	579	+311	10.49	6.68	1.74	+2.07
	June 1-8	21-22	8	1000	228	361	+411	651	176	310	+165	9.30	6.02	1.40	+1.88
	June 9-12	23	8	1000	272	370	+358	308	105	208	- 5	8.64	5.71	1.30	+1.63

\* Abortion March 7.

† Vigantol during Periods 13-23.

larly between 8.12 and 9.73 mgm. per cent, while inorganic phosphorus ranged between 3.45 and 4.09 mgm. per cent. Thus, all the serum inorganic phosphorus values were normal, and most of the serum calcium values were within normal limits. Phosphatase was normal throughout.

Though this patient showed more marked anatomical evidence of previous vitamin D deficiency (osteomalacia, tetany and cataract) than the two preceding subjects, her metabolic behavior exemplified a greater degree of conservatism in that her serum calcium and phosphorus were maintained within normal limits, and her calcium balance on high intake was on the average somewhat higher. This more conservative behavior was most likely the result of the limited supply of vitamin D received prior to the studies. However, this supply was inadequate to enable her to maintain a balance on low calcium intake and to eliminate significant amounts of calcium in the urine. In other words, there was room for improvement in her metabolic behavior, as in Case 3,

if a more adequate supply of vitamin D had been available to her.

*Case 7, Mrs. W. E. T.* Similar to the foregoing case, this was one of mild osteomalacia, cataract and tetany of many years' duration. Likewise, this patient received calcium and cod liver oil prior to the metabolic observation for the treatment of her tetany, so that her serum calcium and phosphorus were within normal limits and her metabolic behavior was conservative by the time the studies were begun (Table III). She was given for the first 2 periods a low calcium-high phosphorus diet, and successively for 2 periods each, two diets similarly low in calcium but progressively lower in phosphorus. On the low calcium intake (142 to 205 mgm. per day) the average balance was slightly positive and a considerable proportion of the calcium output was in the urine, showing that vitamin D action was operative. When this series of diets was repeated, but with the calcium intake raised to 1000 mgm. a day (Periods 7 to 12), the average daily bal-

ance was 195 mgm., and the urinary calcium, though smaller in relation to the total output, was still considerable, showing that her response to high intake was fairly satisfactory by reason of the prior vitamin D store. However, that this was not the best performance of which the patient was capable was demonstrated by the observations during the subsequent 11 periods in which vitamin D in daily doses of 1 cc. of Vigantol, or 12,000 international units, was given. The first 6 periods on vitamin D therapy were on low calcium regimen (Periods 13 to 18), and no obvious difference was noted in the calcium balance, but, subsequently, during Periods 19 to 23, while on high calcium diet, definite changes took place. Not only did the average daily retention improve to 386 mgm., but also the urinary calcium increased greatly. The urinary calcium averaged 221 mgm. per day, amounting to 36 per cent of the total output, signifying that intestinal absorption of calcium had improved so that much more calcium was absorbed than could be retained.

Phosphorus balances varied not only with the levels of phosphorus and calcium intake, but also with the state of vitamin D nutrition. All the high phosphorus periods (1 to 2, 7 to 8, 13 to 14 and 19 to 20) were associated with considerable positive balance, especially in periods of high calcium intake and after Vigantol therapy. In periods of moderate phosphorus intake (Periods 3 to 4, 9 to 10, 15 to 16 and 21 to 22), the balances, which were negative prior to vitamin D therapy, became positive afterwards. Low phosphorus periods showed slightly negative balances, the degree of phosphorus loss remaining uninfluenced by the high level of calcium intake or by the vitamin D therapy.

Serum calcium, fairly normal to start with, tended to fall as studies progressed until vitamin D was given. After this it slowly returned to the initial value. A more definite rise occurred in the level of serum inorganic phosphorus after Vigantol administration.

This patient, though clinically similar to the preceding patient, was somewhat different in metabolic behavior, in that urinary calcium persisted in significant amounts, indicating the presence of a greater store of vitamin D. However, that this store was not the optimum was shown by the improvement in calcium balance, the increase in

urinary calcium and the rise in serum phosphorus subsequent to Vigantol administration.

*Comment.* The four patients in this group are united by the presence of a mild degree of skeletal osteoporosis, but they vary in their metabolic behavior by reason of the varying store of vitamin D. Cases 4 and 5, though presenting less anatomical evidence of vitamin D deficiency than Cases 6 and 7, were nevertheless more deficient in vitamin D, as shown by the metabolic behavior at the time of observation. Obviously, anatomical evidence and metabolic behavior may not necessarily correspond with each other at a given moment. The former is the result of the extent and duration of previous vitamin D deficiency, and once the skeleton is decalcified to an extent to be appreciable by roentgenologic examination, a long period of replenishment is required to eradicate the physical evidence of disease. Some of the evidence, such as cataract, may remain permanently, even if the skeletal lesion is all repaired. On the other hand, metabolic behavior is dynamic, readily influenced by such small amounts of vitamin D as may be introduced by involuntary exposure to sunlight, inclusion in the diet of such items as eggs (11), or the use of limited amounts of cod liver oil for medication, as in Cases 6 and 7. Such factors, often neglected or unrecognized, may make no difference to an individual with an abundant store, but they exert a corrective influence on the metabolic behavior of a patient with vitamin D deficiency, and therefore must be taken into account in interpreting the metabolic data obtained. Furthermore, such vitamin D supply is usually small and, if not continued, can easily be depleted so that metabolic evidence of vitamin D deficiency appears after a varying number of periods of conservative behavior. This was true of Cases 4 and 6 and would have been true of Case 7, had vitamin D been withheld from her. Case 5 was probably deficient in vitamin D from the beginning of the metabolic observations.

Therefore, as a group, these patients all showed mild but definite osseous evidence of previous vitamin D deficiency, but at the time of observation the metabolic behavior indicated a greater current deficiency of the vitamin in Cases 4 and 5 than in Cases 6 and 7, in which some cod liver oil had been given prior to the observations. In severe deficiency (Case 4), high calcium may be of no

avail in promoting sufficient calcium gain for the fetal needs, although in moderate deficiency (Case 6), it is capable of doing so. With adequate vitamin D supply, high calcium intake will enable the patient not only to take care of the demands of pregnancy, but also to store enough calcium for the reparation of her depleted skeleton. Thus, in the treatment of this group of patients, adequate vitamin D therapy, as well as high calcium intake, is necessary.

Comparison of this group of patients with mild skeletal decalcification with the previous group

without bone lesions shows no essential differences in metabolic behavior except insofar as they are related to the state of vitamin D nutrition. In the first group, absence of bone lesions is associated with an early or a mild grade of vitamin D deficiency, if it is present at all. On the other hand, in the second group where bony decalcification is already recognizable, usually severer grades of vitamin D deficiency are present if not previously treated. Whatever metabolic differences may exist between the two groups are to be accounted for by the differences in vitamin D store

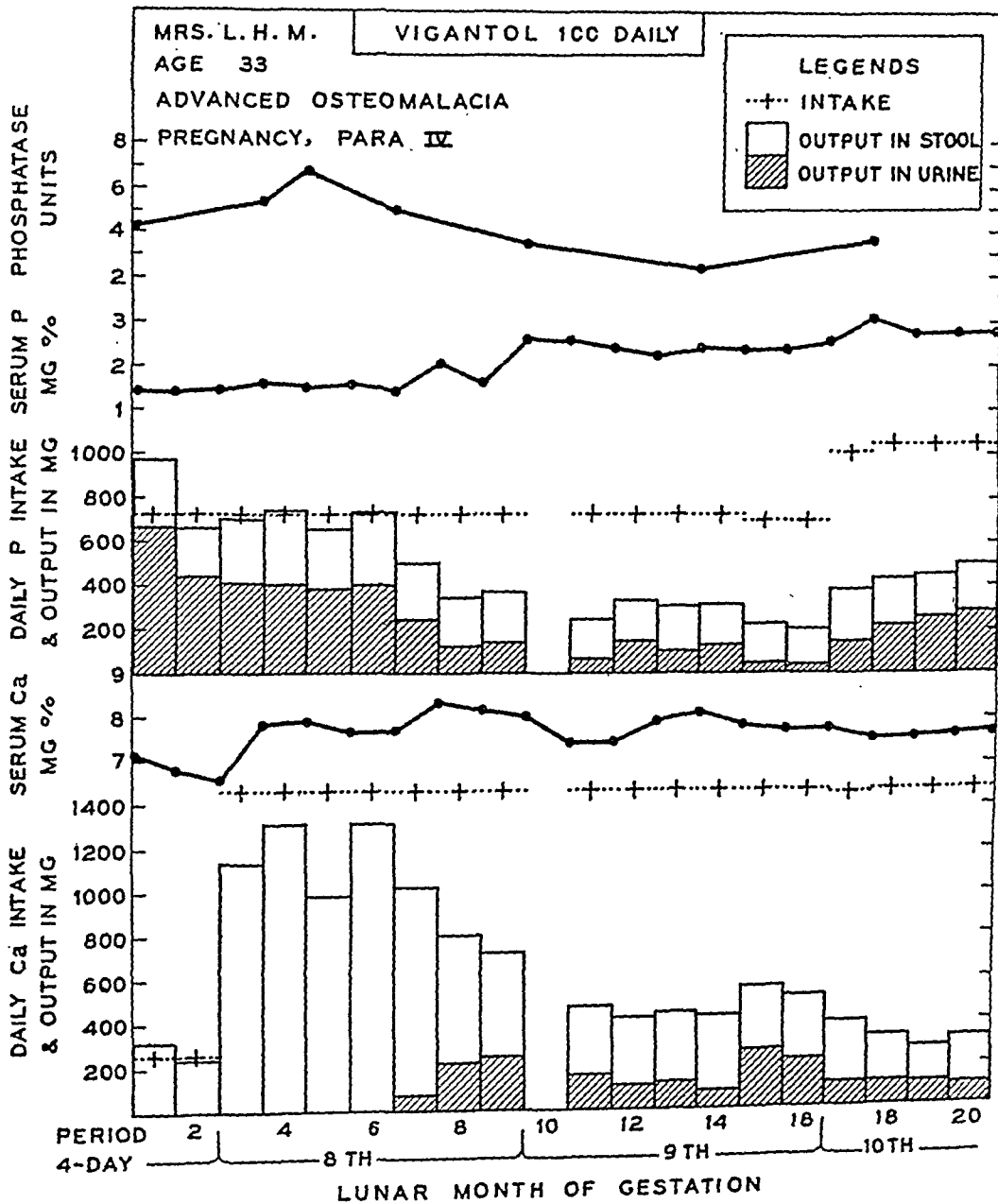


FIG. 3. CASE 8. CALCIUM AND PHOSPHORUS METABOLISM IN ADVANCED OSTEOMALACIA SHOWING REMARKABLE MINERAL RETENTION AFTER VITAMIN D ADMINISTERED DURING THE LATTER PART OF PREGNANCY

TABLE IV

## Group 3. Advanced osteomalacia. Calcium, phosphorus and nitrogen metabolism

Case	Date	Period 4-day	Stage of gestation	Calcium, average daily				Phosphorus, average daily				Nitrogen, average daily			
				Intake	Urine	Stool	Balance	Intake	Urine	Stool	Balance	Intake	Urine	Stool	Balance
8 L.H.M.	1938-39		Lunar months	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	grams	grams	grams	grams
	November 20-27	1-2	7	273	5	274	- 6	724	556	265	- 97	10.39	8.86	2.00	-0.47
	28-December 9	3-5	8	1473	3	1147	+ 323	724	406	296	+ 22	10.39	7.05	1.55	+1.79
	December 10-25	6-9*	8	1473	140	835	+ 498	724	231	257	+236	10.39	6.42	1.63	+2.34
	30-January 14	11-14	9	1473	125	327	+1021	725	114	188	+423	6.87	4.57	1.24	+1.06
	January 15-22	15-16	9	1471	256	294	+ 921	694	42	178	+474	9.10	5.11	1.34	+2.65
	23-February 7	17-20	10	1468	110	225	+1133	1030	231	214	+585	9.10	5.87	1.54	+1.69
9 W.H.S.	1938-39														
	December 26-January 10	1-4	9	1483	60	1268	+ 155	832	405	379	+ 48	10.52	7.47	1.58	+1.47
	January 11-14	5†	10	1438	123	1360	- 45	914	371	381	+162	10.80	7.45	1.77	+1.58
	15-February 3	6-10	10	1398	181	893	+ 324	878	337	282	+259	10.33	6.68	1.70	+1.95
10 Y.W.L.	1934-35														
	September 18-October 3	1-4	3-4	304	6	124	+ 174	974	436	285	+253	10.40	8.12	0.94	+1.34
	October 4-15	5-7	4	458	0	179	+ 279	919	364	324	+231	6.88	5.91	0.93	+0.04
	16-23	8-9	4	118	4	211	- 97	634	281	404	- 51	8.16	5.13	1.35	+1.68
	24-November 4	10-12	5	88	1	65	+ 22	476	240	264	- 28	6.08	3.65	1.19	+1.24
	November 5-20	13-16	5	126	0	97	+ 29	1041	466	414	+161	12.00	8.31	1.28	+2.41
	21-28	17-18	6	1948	233	808	+ 907	323	22	194	+107	9.54	5.98	1.05	+2.51
	December 3-10	20-21	6	1937	13	1384	+ 540	1041	126	494	+421	12.00	8.40	1.13	+2.47
	11-18	22-23†	6	1937	23	1034	+ 880	1041	140	403	+498	12.00	8.38	0.93	+2.69
	19-30	24-26	7	1937	91	888	+ 958	1041	42	468	+531	12.00	8.41	1.19	+2.40
	31-January 15	27-30	7	1937	119	797	+1021	1041	12	422	+607	12.00	8.77	1.22	+2.01
	January 16-23	31-32	8	1937	92	990	+ 855	1041	52	470	+519	12.00	8.82	1.23	+1.95
	February 5-12	36-37	8	1945	202	1030	+ 713	1108	12	658	+438	12.16	8.55	1.52	+2.29
	13-28	38-41	9	1945	202	1086	+ 657	1108	12	736	+360	12.16	9.33	1.42	+1.41
	March 1-12	42-44	9	1943	81	1158	+ 704	1091	165	515	+411	12.00	8.89	1.21	+1.90
	13-24	45-47	10	1945	51	1047	+ 847	1108	214	429	+465	12.16	9.22	0.87	+2.07
	25-April 9	48-51	10	1945	95	1472	+ 378	1108	275	605	+228	12.16	9.24	1.22	+1.70

\* Vigantol during Periods 6-13.

† Vigantol in this period.

‡ Vigantol during Periods 22-51.

rather than by the presence or absence of slight bony rarefaction.

### Group III. Advanced osteomalacia

*Case 8, Mrs. L. H. M.* This subject, aged 33 years, was admitted for study at the seventh month of her fourth pregnancy. She had severe osteomalacia with symptoms dating back eleven years, which was shortly after the birth of her first child. The metabolic data of 20 periods are presented in Figure 3 and Table IV. In the first 2 periods on an intake of 273 mgm. of calcium per day, the output, all in the stools, almost balanced the intake. Beginning with Period 3, the intake was raised to 1473 mgm. daily. During the first 3 periods on the augmented intake, there was, on the average, a daily retention of 323 mgm., or 22 per cent of the intake. This degree of retention may not be abnormally low for a person with a normal skeleton, but for a patient like this with such extensive bony decalcification, together with absence of urinary calcium and low serum calcium and phosphorus, it indicates poor intestinal absorption or severe vitamin D deficiency. The correctness of this interpretation is shown by

her response to Vigantol therapy which was given from Period 6 to 13. From Period 7 onward there was a progressive decrease of stool calcium and, at the same time, the appearance of a considerable amount of calcium in the urine. The average daily retention from Period 11 to 20 was over 1 gram or 70 per cent of the intake. If the last 4 periods, in which the phosphorus intake was raised, were considered alone, the retention averaged 1133 mgm. a day or 77 per cent of the intake. The amount retained would enable the patient not only to meet the requirements of pregnancy, but also to repair her depleted skeleton. Her symptoms were considerably improved.

Phosphorus balances were generally parallel with calcium balances. Serum calcium, 6.58 to 7.18 mgm. per cent during the low calcium periods, was raised to a maximum of 7.90 mgm. per cent during high calcium periods. After Vigantol administration, a slight further rise occurred, but the highest figure reached was only 8.36 mgm. per cent. Serum inorganic phosphorus, 1.45 mgm. per cent to start with, remained at this level until vitamin D administration, after which it showed steady elevation, the maximum being

3.06 mgm. per cent. Serum phosphatase, 4.2 to 6.70 Bodansky units per 100 cc., was lowered to 2.35 to 3.54 units after Vigantol therapy.

This patient, who had a severe osteomalacia, showed evidence of vitamin D deficiency in the early part of the metabolic observations. With vitamin D therapy there resulted remarkable calcium and phosphorus retention. Over and above what was required by the fetus, a substantial part of the retained minerals must have been deposited in the depleted skeleton. In this case, serum calcium and phosphorus failed to rise to perfectly normal levels in spite of adequate vitamin D therapy. Thus it seemed as if the urgent requirement of the skeleton, as well as of the fetus, had to be fulfilled at the expense of the serum concentration of these elements.

*Case 9, Mrs. W. H. S.* This patient, aged 29 years, was observed for 10 four-day periods during the last part of her fourth pregnancy. While the history of osteomalacia and tetany had been of eleven years' duration, and bony deformities were marked, the degree of skeletal rarefaction was not as extensive as in the preceding case, partly on account of previous treatment. As shown in Table IV, the average calcium balance on an intake of 1483 mgm. per day during the first 4 periods was 155 mgm., or approximately 10 per cent of the intake. The poor calcium retention, together with low serum inorganic phosphorus and relatively low calcium, indicates definite vitamin D deficiency. Vitamin D in the form of Vigantol 5 cc. daily was given for four days during Period 5. The response during the subsequent 5 periods consisted of an increase of the average calcium retention to 324 mgm. per day, or 23 per cent of the intake, and a considerable increase of urinary calcium. The response, however, was probably not the best of which the patient was capable in view of the unusual manner in which vitamin D was given. Large doses of Vigantol given for a few days might not be as efficient as smaller doses spread over a longer period of time, although single massive doses of vitamin D have been claimed to be effective in the treatment of rickets (12). It is possible that this patient with a lesser degree of skeletal mineral depletion might not require a higher degree of calcium retention than she showed. However,

this explanation is not likely, because patients with slight skeletal decalcification (Case 7), or without obvious bone lesions (Case 3), exhibited better retention of calcium under adequate vitamin D therapy.

Retention of phosphorus corresponded with that of calcium. Serum calcium was slightly but definitely raised and inorganic phosphorus was markedly elevated after Vigantol therapy.

*Case 10, Mrs. Y. W. L.* This woman of 43 years of age with severe osteomalacia of four years' duration was observed continuously from the third to the tenth month of her fifth pregnancy. For a year previously she went through detailed metabolic studies during which Vigantol 1 cc. daily (12,000 international units of vitamin D per cc.) was given for forty days (ending January 28, 1934) with considerable improvement in metabolic behavior, as well as clinical symptomatology. Observations during the present pregnancy were begun on September 19, 1935, on a diet containing 304 mgm. calcium per day (Figure 4 and Table IV.) On this diet (Periods 1 to 4) more than half of the intake of calcium was retained. This was true of the next diet containing 461 mgm. calcium per day (Periods 5 to 7), indicating satisfactory circumstances. Even when the diet calcium was reduced to 88 to 126 mgm. per day (Periods 8 to 16), balances, on the whole, were even, showing the remarkable power of conservation of calcium in a patient with osteomalacia when a prior store of vitamin D had been present.

From Period 17 on, the calcium intake was raised to 1959 mgm. per day. During the first 2 periods of high calcium intake, associated with very low phosphorus intake, the calcium retention averaged 907 mgm. per day, or 47 per cent of the intake, and the urinary calcium 233 mgm. per day, or 22 per cent of the total output. In distinct contrast were the results of Periods 20 and 21, during which both calcium and phosphorus intakes were high. Here the calcium retention was not as good and the urinary calcium was negligible. It was thought that at this point the patient might be showing an early vitamin D depletion. Therefore, Vigantol 2 cc. daily was started from Period 22. Subsequently, the calcium balances showed slight improvement and the urinary calcium gradually returned (Periods 22 to 32). Likewise, the phosphorus balances in-



creased correspondingly, mainly at the expense of urinary phosphorus.

In view of the presence of anemia, ferric ammonium citrate, 6 grams daily, was given during Periods 34 to 41. The anemia did not respond to the iron therapy; both calcium and phosphorus balances were adversely affected by it. The absorption of phosphorus seemed to be particularly impeded by the presence of large amounts of iron in the intestinal tract, probably on account of the formation of insoluble ferric phosphate. Since the absorption of phosphorus was impaired, the calcium balance would decrease mainly on account of a shortage of phosphorus for simultaneous deposition in the bone; hence the undeposited calcium was eliminated in the urine. The discontinuation of iron administration in Period 42 was followed by improved balances both in calcium and in phosphorus, and by decreasing amounts of calcium and increasing amounts of phosphorus in the urine.

The last 4 periods prior to delivery, however, were associated with poorer retention of both calcium and phosphorus. The explanation was not clear, although the discontinuation of hydrochloric acid administration, which had been given during Periods 39 to 45, might conceivably have removed a factor that promoted absorption. An alternative would be that with prolonged high calcium intake the skeletal store was gradually being replenished, rendering mineral retention less urgent. In support of this supposition, there seemed to be a slight general trend toward decreasing retention throughout the periods of high calcium intake.

Both serum calcium and inorganic phosphorus were within lower limits of normal at the commencement of the observations. Serum calcium varied but slightly except for a tendency to decrease during periods of low calcium intake, and a tendency to increase after vitamin D addition and after iron therapy. Serum inorganic phosphorus fluctuated more widely. In general, it varied directly with the phosphorus intake. While the latter was maintained on a constantly high intake, vitamin D administration was associated with a definite rise and iron therapy with a distinct lowering of serum phosphorus.

There are several points of interest in this patient with advanced osteomalacia. First, while

vitamin D was operative a minimal intake of calcium was associated with an even balance, and a high intake with a retention of 40 to 50 per cent. Second, as observations proceeded, there was a tendency to a decreasing retention. This was considered to be related to a gradual replenishment of the skeletal store rather than to any interference attributable to later stages of pregnancy. Finally, the adverse effects of iron on calcium and phosphorus balances deserve attention. While both calcium and phosphorus retention may be reduced under iron therapy, serum phosphorus may fall with a rise in serum calcium. This phenomenon has been utilized in the treatment of hypocalcemia and hyperphosphatemia associated with chronic advanced renal insufficiency (13).

*Comment.* The three patients in this group, though they were alike in showing marked skeletal decalcification, deformity and fractures, again varied in their metabolic behavior on admission on account of the varying store of vitamin D acquired prior to the studies. Thus Cases 8 and 9 were deficient in vitamin D, while Case 10 exhibited evidence of a considerable store when the studies were begun. Cases 8 and 10, under adequate Vigantol therapy and high calcium and phosphorus intake, consistently showed a retention of calcium and phosphorus considerably over and above the requirements of pregnancy, proving that in osteomalacia it is possible for the patients under such a regimen to gain sufficient minerals for the skeletal reparation as well. The mineral retention in Case 9 was not as much as expected, probably due to the inefficient manner of administering vitamin D. When large amounts of calcium and phosphorus are required for the growth of the fetus as well as for the replenishment of the depleted skeleton of the mother, as in Case 8, serum calcium and phosphorus may fail to rise to perfectly normal levels in spite of adequate vitamin D and high mineral intake.

The behavior that may be said to characterize this group of patients with extensive bone involvement consists of more marked metabolic evidence of vitamin D deficiency when untreated, unusually high retention of calcium and phosphorus under high mineral and vitamin D intake, and occasional failure of the serum calcium and phosphorus to rise to normal in spite of such therapy.

## DISCUSSION

Quantitative measurements of calcium and phosphorus metabolism in osteomalacia during pregnancy of the type here presented are not available in the literature except for the two cases which have been briefly reported by us (14). Such data are important in evaluating the rôle of pregnancy in the pathogenesis of osteomalacia in view of the frequent association of the two. A comparison of the metabolic behavior during pregnancy of patients with severe or mild osteomalacia with that of subjects showing no skeletal lesions reveals no essential differences. With adequate vitamin D supply and moderately high intake of calcium and phosphorus, subjects of various skeletal condition have no difficulty in retaining sufficient amounts of the minerals for the needs of gestation. In fact, under similar regimen, osteomalacic patients tend to retain more calcium and phosphorus in an attempt to replenish the depleted maternal store, as well as to provide for fetal growth. In other words, there is no inherent inability on the part of osteomalacic patients during pregnancy to utilize calcium and phosphorus in the midst of plenty of these minerals and vitamin D. Whatever metabolic defect they may show during gestation results from limited vitamin D and mineral supply just as it does during reproductive rest.

Furthermore, in contrast to lactation, where the physiological activity is such that mineral requirement has to be increased much above what is secreted in the milk, even in the presence of adequate vitamin D, there is no such factor in pregnancy. Cases 9 and 10 were subsequently observed during lactation and reported on as Cases 1a and 4a in paper IX of this series (2). In both instances, the mineral balances were much less favorable during lactation than during pregnancy under similar conditions of adequate vitamin D and high calcium intake. Moreover, in Case 5, where the termination of pregnancy was not followed by lactation, mineral retention did not improve after delivery, again showing that pregnancy itself has no excessively deleterious influence on mineral retention.

However, the minimal requirement for fetal growth of 200 mgm. calcium and 100 mgm. phosphorus per day during the last three months of

gestation has to be met through the maternal mineral resources. The usual level of calcium intake in Chinese dietary is approximately 0.337 gram and that of phosphorus 1.2 grams (15), although somewhat higher levels of intake have been recorded by others. Such a level of calcium intake, in the presence of vitamin D, may maintain an individual in balance under ordinary circumstances, but in pregnancy it cannot be expected to yield adequate mineral balance for the fetal requirement. The extent of drain on the skeletal store of the mother will depend upon her state of vitamin D nutrition. If there is adequate vitamin D supply—sometimes from the diet, but usually from sunlight (16)—a variable proportion of the total fetal requirement (21 to 23 grams of calcium) may have to be drawn from the maternal store. This alone may not constitute a serious loss to the mother. On the other hand, in the absence of vitamin D, the mineral loss will be much greater than that imparted to the fetus. Under such circumstances, pregnancy plays an important rôle in the causation of osteomalacia. Moreover, pregnancy is usually followed by prolonged lactation, which constitutes a much greater drain upon the maternal skeletal store. Such a reproductive cycle frequently repeated under an inadequate supply of calcium and vitamin D will inevitably lead to the development of osteomalacia.

As to the actual level of calcium that may be considered adequate to meet the needs of gestation, our data do not give a clear-cut answer. In the first subject who was presumably normal from the standpoint of vitamin D nutrition, an intake of 1 gram of calcium was necessary to bring about sufficient retention for the fetal needs. In individuals (Cases 2, 3 and 6) in whom the vitamin D supply was limited, or beginning to be depleted, an intake of 1.0 to 1.3 grams of calcium seemed also adequate for the gestatory requirement. There was evidence that, in the presence of greater supply of vitamin D, they could acquire the same degree of retention on an intake level considerably lower than 1.3 grams (Case 3). However, in severe grades of vitamin D depletion, an intake of 1.3 grams, or higher, of calcium would not maintain the individual in balance (Case 4). These data all go to show that vitamin D is a more important factor than the actual level of



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calcium intake in determining the extent of retention, provided a reasonable amount of calcium is present in the diet. As to phosphorus, its utilization depends a great deal on that of calcium. As Chinese dietaries contain good amounts of phosphorus, adequate calcium retention usually means adequate phosphorus retention. Likewise, there is apparently no difficulty in nitrogen metabolism with usual Chinese dietaries.

A comparison of the data of these subjects showing varying skeletal condition and vitamin D store with those of presumably normal women in pregnancy available in the literature shows greater degree of mineral conservation in our patients. Toverud and Toverud (17) made short periods of observation on thirty Norwegian women living in a home for expectant mothers during the last two to three months of pregnancy. Negative calcium and phosphorus balances were the rule on the usual home diets, but positive balances were sometimes observed after the intake of calcium and phosphorus had been increased to 1.6 to 2.0 grams. Coons *et al.* (5) reported the results on 2 groups of women, one in Chicago, the other in Oklahoma. With an average intake of 1.4 grams calcium and 1.6 grams phosphorus, adequate retention for gestatory needs occurred in the Southern women, but not in the Chicago women, the difference being attributed to the influence of sunshine. Macy and Hunscher (4), from a compilation of data in the literature on mineral utilization during pregnancy, concluded that during the last three months an intake of 1.4 to 1.5 grams of calcium and 2 grams of phosphorus was necessary to secure adequate retention for the demands of pregnancy. On the other hand, in our subjects 1.0 to 1.3 grams of calcium seemed adequate for the requirements of pregnancy, even when the supply of vitamin D was limited. With optimum vitamin D nutrition calcium requirement may be lowered.

This relative conservatism shown by our patients cannot be entirely due to the depleted skeletal store which would cause calcium to be retained with great avidity because the patients in the first group without obvious osseous decalcification exhibited the same phenomenon. Another important factor seems to lie in the previous level of intake. When the dietary habits accustom the subject to a lower intake, the added requirement

for reproductive activity will be correspondingly lower. Furthermore, vitamin D plays such an important rôle in conserving calcium that its judicious use will make it possible to decrease the usually quoted requirement for a given state of physiological activity. The combination of previous low level of intake and existing vitamin D action probably explains the unusual ability on the part of five of the ten subjects in this series to retain calcium on intakes varying from 88 to 277 mgm. per day (Cases 3, 4, 6, 7 and 10). It is plain, then, that the so-called calcium requirement, contrary to current conception, must be regarded as a variable quantity conditioned by such factors as the prior skeletal store, the previous dietary custom, and the state of vitamin D nutrition.

#### SUMMARY

Data on calcium, phosphorus and nitrogen metabolism during the latter part of pregnancy were obtained on ten subjects showing various states of skeletal store and vitamin D nutrition. Given an adequate supply of vitamin D and calcium, patients with osteomalacia showed no inherent inability to retain minerals during pregnancy, compared with those with no skeletal depletion. The added requirement during gestation, unlike that in lactation, did not seem to go beyond fetal needs. However, such needs had to be filled at the expense of the maternal tissue, if the supply of vitamin D and minerals was inadequate. Under such circumstances, pregnancy plays an important pathogenetic rôle in osteomalacia inasmuch as it hastens the skeletal demineralization. While high calcium intake tends to ameliorate the effects of vitamin D deficiency, the latter conserves calcium. Of the two, vitamin D is probably more important, provided a reasonable level of calcium intake is available. The calcium requirement during pregnancy is conditioned by the prior skeletal store, the previous dietary intake, and the state of vitamin D nutrition.

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# OBSERVATIONS ON THE EFFECT OF STREPTOCOCCAL UPPER RESPIRATORY INFECTIONS ON RHEUMATIC CHILDREN: A THREE-YEAR STUDY<sup>1</sup>

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The relationship of beta hemolytic streptococci to the etiology of rheumatic fever has long been a controversial subject. Although many students (1) of this disease are of the opinion that both the initial attack and subsequent recurrences of rheumatic fever are usually preceded by streptococcal upper respiratory infections, others (2) believe that this association is merely accidental. At the present time, there is no adequate explanation of the exact rôle played by streptococci in the etiology of rheumatic fever. On the other hand, the association between streptococcal upper respiratory infections and rheumatic fever seems too constant to be completely disregarded.

It is obvious that many factors which cannot be adequately studied in a general hospital or in ambulatory rheumatic patients enter into this problem. Studies of this kind can be carried out more satisfactorily in sanatoria where the patients are relatively isolated and are under careful medical supervision for long periods of time. Since the severity of both streptococcal upper respiratory infections and rheumatic fever is known to vary from year to year, it was thought that a comparison of the results obtained during three successive winters would yield more reliable information than observations limited to a single year.

## PLAN OF STUDY

The same methods and procedures were used during three successive years, 1937 to 1940.

### *Type of institution*

Irvington House, a sanatorium for rheumatic children, is situated in the country, 20 miles from New York City. The children were admitted from the cardiac clinics of various hospitals in Greater New York. Children who had had one or more attacks of polyarthritis or carditis without marked cardiac damage were selected. With a few exceptions, children with histories of "pure" chorea without polyarthritis or carditis were excluded. New

cases were admitted only during the summer and fall months. Each year 108 suitable patients, consisting of new children and children remaining longer than one winter, were assembled by the first of December. This group then remained in the institution until the end of May or longer.

The children lived and went to school in the same building. They had no contact with other children and each child was permitted only two adult visitors every six weeks. The group consisted of 66 girls and 42 boys varying in age from 6 to 15 years.

### *Routine procedures*

Rectal temperatures and pulse rates were taken three times daily. Leukocyte counts, hemoglobin estimations, and erythrocyte sedimentation rates were done routinely every three or four weeks, or more often when necessary. Children who had symptoms of upper respiratory infections were put to bed and isolated in cubicles, or when possible, in separate rooms. Leukocyte counts were taken on the first or second day of illness.

### *Bacteriological procedures*

Throat cultures to determine the presence of Group A beta hemolytic streptococci were taken routinely once a week on every child throughout the year. Additional cultures were taken on two successive days on children who developed symptoms of any kind. Swabs were streaked on freshly prepared 5 per cent sheep blood agar plates (3). After eighteen-hour growth, hemolytic colonies were re-streaked and fished to broth. Attempts were made to type all Group A beta hemolytic streptococci isolated. Both the slide agglutination method of Griffith (4) and the precipitin reaction of Lancefield (5) with "M" extracts were used. In most instances, cultures were considered to be successfully typed only when the findings obtained by slide agglutination could be confirmed by the precipitin reaction. Many strains were isolated which could not be typed by either method. In several instances, agglutinating and precipitating sera were prepared by immunizing rabbits with these cultures.

Antistreptolysin O titers (6) were determined every three to six weeks, and more often following upper respiratory infections or during rheumatic activity. Antistreptolysin S titers (6) were not determined.

### *Definitions*

It has been noted by several observers that the clinical symptoms of the streptococcal upper respiratory infec-

<sup>1</sup> This work was aided by a grant from The Commonwealth Fund.

tions preceding the development of rheumatic recurrences may be extremely mild and therefore can be easily overlooked.

The cases of streptococcal upper respiratory infections reported in this study were in most instances clear-cut. The children complained of sore throat and the pharynx usually appeared inflamed. Occasionally exudate was present. Most of the patients had fever and elevated white blood counts. Throat cultures taken on the first or second day of illness showed varying numbers of Group A beta hemolytic streptococci of a type which had not been present previously.

In certain instances, however, especially during the winter of 1937 to 1938, it was difficult to decide whether a child really had an upper respiratory infection or had merely become a streptococcus carrier. A few children who had neither complaints nor symptoms, but whose white blood counts showed a definite rise coincident with the appearance of streptococci in their throat culture, or whose antistreptolysin O titer rose three to six weeks later, were considered to be cases of upper respiratory infection.

Children in whom the appearance of streptococci in their routine throat cultures was not accompanied by symptoms, or by a rise in their white blood counts or in their antistreptolysin O titer, were considered to have become carriers, either temporary or chronic.

It is well known that the course of rheumatic fever varies greatly. In some children, evidence of low-grade activity persists for months and years. This type of case is generally described as continuous or polycyclic. Slight exacerbations of the symptoms may occur from time to time in these children without apparent precipitating cause. Other children are subject to severe, acute attacks but between attacks clinical or laboratory signs of rheumatic activity are not demonstrable. It is in this monocyclic type of case that factors tending to precipitate rheumatic recurrences can be studied most satisfactorily.

### *1. Streptococcal upper respiratory infections occurring during the winter of 1937 to 1938*

During this winter, 12 of the 108 children developed upper respiratory infections associated with Group A beta hemolytic streptococci of a single type. The organisms isolated from these cases did not belong to any of the known serological types of streptococci. Agglutinating and precipitating sera were therefore prepared by immunizing rabbits with a culture, C51, isolated from one of these patients (Case 2, Table I). By means of slide agglutination and precipitin tests, the organisms isolated from the other 11

patients were shown to belong to the same serological type as C51.<sup>2</sup>

The symptoms produced by this infection were mild. The 12 cases appeared sporadically over a period of six months, the first occurring in December 1937 and the last in May 1938. Prior to this infection, 2 of the 12 children had continuously elevated erythrocyte sedimentation rates, and the rheumatic infection in these 2 individuals therefore was not considered to be quiescent. Neither of these 2 children had exacerbations of their symptoms following the upper respiratory infection. The remaining 10 children had not had signs of rheumatic activity for six months or longer. Of these 10 children, 6 developed rheumatic recurrences after latent periods varying from nine to eighteen days. Five of the 6 children who developed rheumatic recurrences showed a prompt rise in antistreptolysin O titer. Three children who did not develop recurrences also showed a definite rise in titer (Cases 5, 6 and 9). The data in regard to these 12 cases are presented in Table I.

Ten children became carriers of streptococcus C51. None of them had rheumatic recurrences. Except for 3 children who showed continuous low-grade activity, none of the other 83 children in the institution who did not contract infections with streptococcus C51 developed symptoms of rheumatic activity.

### *2. Streptococcal upper respiratory infections occurring during the winter of 1938 to 1939*

None of the children developed streptococcal upper respiratory infections during December and January. Beginning in February 1939 and continuing through June, 32 children developed upper respiratory infections associated with Group A beta hemolytic streptococci of a single type, Type 4. The majority of these infections were definitely more severe than those associated with streptococcus C51 which had occurred during the previous winter. Many of the children had fever of 101 to 103° F. for two or three days. The

<sup>2</sup> Nine of these cultures and some agglutinating serum were sent to Dr. Fred Griffith in England. He agreed that these strains did not belong to any of the known Group A streptococcal types and that they were serologically identical.

TABLE I

Data on streptococcal upper respiratory infections with streptococcus C51 and rheumatic sequelae during the winter 1937 to 1938

Case number	Patient			Previous rheumatic attacks and age at each	Date	Clinical findings	Strep-tococcus C51, number of colonies	Latent period	Blood			
	Name	Age	Sex						Date	White blood cells	Sedimentation rate*	Anti-streptolysin O titer
1	I.R. 3264	years 8	F	6 Ch† 7 Ch	December 27, 1937 January 12, 1938	Pharyngitis. No fever. Mild carditis.	few	days 16	November 15 December 10 December 27 January 13 January 19 January 24 January 30 February 4 February 20	5,000 8,800 8,800 7,100 6,500	mm. 2 5 31 30 21 4 6	units 100 80 100 100 100 100
2	A.C. 3236	11	M	3 P and C 5 P and C 6 C 8 P and C 9 P and C	January 3, 1938 January 15, 1938	None. Severe carditis, second degree heart block, nodules, erythema.	50	12	December 27 December 30 January 10 January 13 January 16 January 19 January 23 January 30 February 12 March 5	7,800 11,500 22,700 24,500 15,200 9,900	1 6 24 30 30 31 30	80 165 200 250 300 300
3	C.F. 3269	10	M	7 Ch 7 P 7 P and C 8 Ch	January 7, 1938	Pharyngitis. Fever 100 to 102.6° for one day. No rheumatic sequelae.	15		December 21 January 8 January 10 January 21 January 24 February 5 February 7 February 11	7,600 12,400 7,800 5,400 7,800	10 6 9 21 7	80 100 100 100 100
4	W.C. 3294	11	M	7 P	January 27, 1938	Pharyngitis. Fever 100 to 101.4° for two days. No rheumatic sequelae.	75%		January 18 January 23 January 27 January 28 February 4 February 11 February 20 March 1 March 14	4,400 7,600 4,850 4,800 5,200	6 28 28 22 17 13 13 6	50 50 50 50 50 60
5	G.H. 3278	13	F	9 P and C	February 19, 1938	Pharyngitis. Fever 100.4 to 102.6° for two days. No rheumatic sequelae.	75%		February 13 February 16 February 19 February 21 February 22 February 26 March 12 March 21 April 7 April 14 April 20 April 25	9,300 11,600 6,000 10 10 6,400 9,500 8,300	3 20 10 9 2 6	50 50 100 100 250 200 100
6	H.S.† 3283	13	F	5 Ch 7 Ch 10 Ch 11 Ch	February 22, 1938	Pharyngitis. Fever 100.4° for one day. Mild sinusitis after twenty-eight days, left antrum washed. No rheumatic sequelae.	25%		January 22 February 12 February 16 February 22 February 26 February 28 March 5 March 12 March 22 March 28 March 30 April 7 April 11 April 14	8,200 14,800 9,900 12,800 8,000 7,500	28 23 18 22 21 34 32 28 29	80 60 100 100 200 165

\* Erythrocyte sedimentation rate, Wintrobe-Landsberg method, values over 10 mm. are abnormal.

† The following abbreviations are used: Ch Chorea

P Polyarthritides

C Carditis

Jt P Joint pains

H D Heart disease diagnosed.

‡ This patient had continuous low grade activity (elevated sedimentation rate), no exacerbation was observed.



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In certain instances, however, especially during the winter of 1937 to 1938, it was difficult to decide whether a child really had an upper respiratory infection or had merely become a streptococcus carrier. A few children who had neither complaints nor symptoms, but whose white blood counts showed a definite rise coincident with the appearance of streptococci in their throat culture, or whose antistreptolysin O titer rose three to six weeks later, were considered to be cases of upper respiratory infection.

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patients were shown to belong to the same serological type as C51.<sup>2</sup>

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None of the children developed streptococcal upper respiratory infections during December and January. Beginning in February 1939 and continuing through June, 32 children developed upper respiratory infections associated with Group A beta hemolytic streptococci of a single type, Type 4. The majority of these infections were definitely more severe than those associated with streptococcus C51 which had occurred during the previous winter. Many of the children had fever of 101 to 103° F. for two or three days. The

<sup>2</sup> Nine of these cultures and some agglutinating serum were sent to Dr. Fred Griffith in England. He agreed that these strains did not belong to any of the known Group A streptococcal types and that they were serologically identical.

pharynx in most of the cases looked definitely inflamed and the white blood count was elevated with counts ranging from 13,000 to 24,000 in a large proportion of the cases. There were 4 cases in February. The outbreak reached its peak in March with 17 cases and declined gradually with 6 cases in April and 5 in May. Eleven of the 32 children showed a definite rise in antistreptolysin O titer. Fourteen children became carriers of streptococcus Type 4.

Aside from the streptococcus Type 4 infections during March, 6 children developed upper respiratory infections with streptococci other than Type 4. The cultural characteristics of the organisms isolated from these 6 cases were so similar that it was thought possible that they might belong to one serological type. However, it was impossible to identify these strains with any of the known types of streptococci. Attempts to produce agglutinating and precipitating sera by immunizing rabbits with one of the cultures isolated from this group of patients were unsuccessful. Although the serological identity of these 6 strains was not proved, they were tentatively considered to represent the same type, designated as 97T.

The symptoms produced in these 6 children were similar to those associated with the streptococcus Type 4 infections; and these two infections could not be distinguished clinically. Three of these 6 children showed a rise in antistreptolysin O titer. One child contracted both the Type 4 and the 97T infections.

None of the 38 children who had these streptococcal upper respiratory infections (32 cases due to Type 4, 6 cases probably due to the group of organisms represented by 97T) developed rheumatic sequelae. Nor did the remaining 70 children who escaped streptococcal upper respiratory infections develop clinical or laboratory signs of rheumatic activity.

### *3. Streptococcal upper respiratory infections occurring during the winter of 1939 to 1940*

Beginning in November 1939 and continuing until May 1940, 39 of the 108 children developed upper respiratory infections associated with Group A beta hemolytic streptococci of a single type, Type 27. Most of these infections were slightly

less severe than those associated with streptococcus Type 4 and streptococcus 97T observed during the spring of 1939, but definitely more severe than those associated with streptococcus C51 occurring during the winter of 1937 to 1938. Following a latent period of twelve to twenty-two days, 8 of 39 children who had upper respiratory infections associated with streptococcus Type 27 developed rheumatic recurrences. The seasonal incidence of these infections and distribution of recurrences are presented in Table II.

TABLE II  
*The seasonal distribution of streptococcus Type 27 upper respiratory infections and incidence of rheumatic recurrences*

Seasonal distribution	Number of cases	Rheumatic recurrences
November.....	5	1
December.....	8	2
January.....	16	2
February.....	3	0
March.....	6	2
April.....	1	1
Total.....	39	8

The data regarding the 8 children who developed rheumatic recurrences are presented in Table III.

The severity of the pharyngitis varied greatly. Two children had no fever and one had slight malaise with a maximum temperature of 100.4° F. on one day. The other 5 had definite febrile reactions accompanied by elevated leukocyte counts. With one exception, the number of streptococci present in the throat was large. After a latent period varying from eleven to twenty-two days following the streptococcal pharyngitis, these 8 patients developed clear-cut rheumatic manifestations. Four of the 8 children showed a rise in antistreptolysin O titer within a month or less following the upper respiratory infection. In 3, a slight or questionable rise occurred after five weeks and in one there was no rise.

Of the 31 children in whom the streptococcal pharyngitis was not followed by rheumatic sequelae, 6 showed a definite rise in antistreptolysin O titer. Three of these 6 children developed cervical adenitis accompanied by a secondary rise in temperature. The remaining 25 children, who escaped rheumatic recurrences, showed no significant rise in antistreptolysin O titer.

TABLE I (continued)

Case number	Patient			Previous rheumatic attacks and age at each	Date	Clinical findings	Strep-tococcus C51, number of colonies	Latent period	Blood			
	Name	Age	Sex						Date	White blood cells	Sedimentation rate*	Anti-streptolysin O titer
7	M.D. 3376	years 14	F	8 P 9 P 10 P	February 23, 1938	Pharyngitis. Fever 100.6° for one day.	12	days	February 13	9,200	mm. 5	units 80
					March 9, 1938	Polyarthrititis.		15	February 25 February 26 February 28 March 6 March 9 March 12 March 16 March 22 March 28 April 16	13,100 8,700 16,600 17,200 11,500 13,300 9,600	15 18 27 19 29 12	80 100 250 333 333 250
8	E.Mc.† 3307	11	F	6 Ch 7 Jt P	March 10, 1938	Pharyngitis. Fever 100.4 to 101° for two days.	few		February 12 February 25 March 5	6,100	16 21	100
						No rheumatic sequelae.			March 12 March 21 March 28 April 3 April 17 April 30 May 6 May 13	8,500 5,500 5,500 5,000	27 29 21 25 21 15 13	100 100 100 165 165
9	R.W. 3381	11	F	9 P and C	March 19, 1938	Pharyngitis. Fever 100.2 to 102.4° for three days.	50%		February 19 March 17	5,900	1 2	50
						No rheumatic sequelae.			March 19 March 21 March 22 March 28 April 10 April 23 April 30	19,800 8,100 8,100 7,800	16 7 1 1 1	60 80 100 80 80
10	F.B. 3382	12	F	7 C	March 28, 1938	Pharyngitis. Fever 100 to 102° for one day.	25		March 14 March 21	6,700	12	100
					April 12, 1938	Polyarthrititis, mild carditis (PR interval prolonged).		15	March 28 April 3 April 6 April 10 April 12 April 16 April 21 April 30 May 6 May 16 May 19	17,400 10,600 9,900 16,500 11,800 11,600 12,600 8,200	17 18 18 31 20 30 34 25	100 100 100 250 250 250 250
11	F.C. 3361	13	F	6 P 7 P 10 P	April 5, 1938	Pharyngitis. No fever.	40		March 4 March 12 April 2	6,900	15 5	100
					April 23, 1938	Carditis, mild (PR interval prolonged).		18	April 5 April 7 April 10 April 17 April 20 April 22 April 23 April 25 April 30 May 4 May 6 May 12 May 19 June 9	8,000 7,000 7,500 10,000 10,000 8,100 6,700 9,400 9,900 4,700	10 12 30 32 17 23 21 10	100 100 100 200 200 165 165
12	R.S. 3110	11	F	5 Jt P 7 H D 8 Jt P 9 C	May 5, 1938	None.	few		April 28	8,500	5	50
					May 14, 1938	Mild carditis.		9	May 5 May 13 May 15 May 16 May 22 May 29 May 31 June 4 June 6 June 9 June 12 June 18 July 2	8,900 13,900 12,800 8,800 9,500 11,500 11,300 7,900	30 31 24 28 21 18 1	60 100 165 200 165 165 100

pharynx in most of the cases looked definitely inflamed and the white blood count was elevated with counts ranging from 13,000 to 24,000 in a large proportion of the cases. There were 4 cases in February. The outbreak reached its peak in March with 17 cases and declined gradually with 6 cases in April and 5 in May. Eleven of the 32 children showed a definite rise in antistreptolysin O titer. Fourteen children became carriers of streptococcus Type 4.

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TABLE III

*Data on streptococcal upper respiratory infections with streptococcus Type 27 followed by rheumatic sequelae in 8 children during the winter 1939 to 1940*

Case number	Patient			Date	Clinical findings	Streptococcus Type 27, number of colonies	Latent period	Blood			
	Name	Age	Sex					Date	White blood cells	Sedimentation rate	Anti-streptolysin O titer
1	I.M. 3513	10 years	F	November 17, 1939  December 6, 1939	Pharyngitis. Fever 103.6 to 101° for three days.  Polyarthrititis, carditis (PR interval prolonged).	75%	19 days	October 17			
								November 6	8,300	1 7	165 165
								November 17	15,200		
								November 20	9,200	30	100
								November 28		31	250
								December 5		29	250
								December 7	13,700		
								December 15	14,800	28	250
								December 22		23	
								January 6		14	250
								January 11	6,500		
								January 14		15	250
								February 1		12	200
								February 10	8,300		
								February 17		6	200
2	H.W. 3457	9	F	December 17, 1939  December 28, 1939	Malaise. Fever 100.4° for one day.  Carditis.	30	11	November 25	5,400		
								December 5		5	100
								December 21		19	100
								December 28	17,000	15	
								January 2	15,700		
								January 4	11,200	32	165
								January 12		33	200
								January 15	10,500		
								January 19		35	250
								January 26		28	200
								January 29	8,900		
								February 2		27	200
								February 19	7,000		
								February 23		23	200
								March 1		16	
3	O.G. 3345	13	F	December 22, 1939  January 8, 1940	Pharyngitis, slight adenitis. Fever 103 to 100.2° for three days.  Polyarthrititis, carditis (PR interval prolonged).	80%	17	December 6		3	40
								December 13	7,800		
								December 23	17,900	28	40
								December 26	10,600		
								December 28	12,500		
								January 6		6	
								January 9	11,500	33	80
								January 15	9,900	33	165
								January 22	10,700	34	250
								January 29		33	250
								January 31	9,200		
								February 12		27	150
								February 19		21	150
								February 26	4,500	18	100

\* This boy's sedimentation rate had been continuously elevated from the date of admission, October 11, 1938, a period of over a year. His rheumatic process, therefore, was never considered entirely quiescent. However, the attack of polyarthrititis was typical and followed the streptococcal pharyngitis after the usual latent period.

TABLE III (continued)

Case number	Patient			Date	Clinical findings	Streptococcus Type 27, number of colonies	Latent period	Blood													
	Name	Age	Sex					Date	White blood cells	Sedimentation rate	Anti-streptolysin O titer										
4	J.C. 3323	years 13	F	January 12, 1940 January 24, 1940	Pharyngitis. No fever. Fever, erythema multiforme, knee painful.	8	days 12	December 26	9,200	2	100										
								January 6		3											
								January 8													
								January 17		1	100										
								January 26		10,100	24	100									
								January 29		10,300											
								January 31		11,400											
								February 2		27	100										
5	R.T.* 3449	11	M	January 22, 1940 February 9, 1940	Pharyngitis. Fever 103.6 to 101.4° for three days. Polyarthrititis.	75%	18	December 11	6,400	27	100										
								January 4		19											
								January 13													
								January 23		18,300	35	100									
								January 24													
								January 26		11,100	24	100									
								January 31		7,900	31										
								February 8													
6	A.D. 3436	13	F	March 25, 1940 April 11, 1940	Pharyngitis. Fever 102.4 to 100.4° for two days. Carditis (PR interval prolonged).	75%	17	February 10	9,400	28	300										
								February 15		32	300										
								February 22													
								February 24		10,900											
								February 27		5,700	32	300									
								February 29			29	250									
								March 21		6,000											
								7		A.B. 3518	11	F	March 26, 1940 April 17, 1940	Pharyngitis. Fever 102.2 to 100.6° for two days. Carditis.	70%	22	March 15	4,400	1	200	
March 9	1	200																			
March 15																					
March 28	12,300	3	165																		
April 1	9,500	13																			
April 4		8																			
April 11																					
April 15	10,300																				
8	A.B. 3518	11	F	April 17, 1940	Carditis.	70%	22	April 18	12,500	19	200										
								April 22		13,600	14	200									
								April 25			21	250									
								May 2		8,900	6	250									
								May 15		5,900											
								9		A.B. 3518	11	F	April 17, 1940	Carditis.	70%	22	April 18	12,500	19	200	
																	April 22		13,600	14	200
																	April 25			21	250
May 2	8,900	6	250																		
May 15	5,900																				

TABLE III (continued)

Case number	Patient			Date	Clinical findings	Streptococcus Type 27, number of colonies	Latent period	Blood					
	Name	Age	Sex					Date	White blood cells	Sedimentation rate	Anti-streptolysin O titer		
8	T.L. 3508	years 13	F	April 19, 1940	Pharyngitis. No fever.	80%	days 20	March 2		mm. 3	units 80		
								April 9		1	60		
								April 15	6,800				
								April 20	8,500				
				May 9, 1940	Carditis (PR interval prolonged), erythema.			April 24	8,900	15			
								May 2	8,850	6	80		
								May 9	10,500	14	80		
								May 13	13,700	34	80		
								May 20	6,700	33	80		
								May 27		32	100		
								May 29	9,500				
								June 10	4,700	17	100		
								June 30		8	80		
								July 10	6,000				

Three children who still had some evidence of rheumatic activity when they were admitted in the fall developed exacerbations during the course of the winter. Twenty-three children who became carriers of streptococcus Type 27 showed no signs of rheumatic activity. None of the remaining 43 children in the institution developed rheumatic manifestations from November 1939 until July 1940.

#### *Comparison of the three epidemic strains isolated 1937 to 1940*

Since the effect of these three outbreaks of streptococcal upper respiratory infections varied greatly in regard to the incidence of rheumatic recurrences, it seemed of interest to compare the three epidemic strains. Therefore, streptococcus C51 (Case 2, Table I), streptococcus Type 4 (Lee) and Type 27 (Case 1, Table III), as well as one of the strains of undetermined type isolated in March 1939, 97T (Tudda), were compared as to capsule formation, colony form, mouse virulence, production of the type specific protein "M" and toxin production.

#### METHODS

**Capsules.** Capsules were studied in India ink preparations with freshly isolated cultures grown from three to five hours in tryptic digest broth. One large loop of culture and one small loop of sterilized India ink were mixed on a plain slide. A coverslip was then pressed

down on the drop so as to make a thin layer. The preparation was examined with an oil immersion lens. Only organisms which appeared to be surrounded by a clear area of approximately 1 mm. or more in diameter were considered to have capsules.

**Colony type.** Broth cultures of recently isolated strains were streaked on freshly prepared 5 per cent rabbit blood agar plates so as to obtain discreet colonies. The plates were sealed with parafilm and incubated for eighteen hours. The watery appearance of typically mucoid colonies was striking. Matt colonies were identified with the aid of a colony microscope.

**Mouse virulence.** One tenth cc. of an eighteen-hour broth culture of recently isolated strains was injected intraperitoneally into mice, weighing approximately 20 grams. If this amount failed to kill, the culture was considered avirulent. Attempts to raise the virulence of C51, Type 4 and 97T by serial mouse passage proved unsuccessful.

**Anti "M" precipitating sera.** Rabbits were immunized with different strains of the epidemic streptococci C51, Type 4, 97T and Type 27 to determine if precipitins for the type specific protein "M" could be produced. With streptococcus C51 and Type 4, sera were obtained which gave positive precipitin reactions with "M" extracts of the homologous types. To date, the preparation of anti "M" precipitating sera with streptococcus 97T and Type 27 has been unsuccessful.

**Toxin production.** Toxin production was tested by the intravenous injection (7) into rabbits of forty-eight-hour toxin prepared in the usual way (8). For purposes of comparison, a forty-eight-hour toxin was prepared at the same time with the standard toxin strain, NY5. It was found that 1 to 2 cc. of the NY5 killed full-grown rabbits regularly in eighteen hours, whereas 10 to 15 cc. of the toxins prepared from the other four strains failed to kill.

The toxin production was further tested on 4 Dick-positive rheumatic children. The NY5 toxin in a dilution of 1:5000 gave positive reactions in all 4 children, whereas the toxins of the four other strains diluted 1:1000 gave irregular results, one child reacting positively and another negatively to the same toxin. In view of the fact that these 4 children were known to have had fairly recent streptococcal upper respiratory infections, it was thought that the positive reactions might be due to sensitivity to streptococcal protein rather than to toxin. These tests, therefore, were considered unreliable.

The results of the comparison of the four strains are presented in Table IV.

TABLE IV  
*Comparison of the epidemic strains of streptococci*

Strain	Year	Number of cases U.R.I.*	Number of rheumatic recurrences	Capsule	Colony	Mouse virulence	"M"	Erythrogenic toxin
C51	1937-1938	12	6	large	large mucoid	negative	+	negative
†Type 4	Spring 1939	32	0	negative	medium matt	negative	+	negative
97T		6	0	large	mucoid	negative	?	negative
Type 27	1939-1940	39	8	large	large mucoid	negative	?	negative

\* U.R.I. = upper respiratory infections.

† Since this paper was accepted for publication, comparative tests on the production of streptolysin O and fibrinolysin by the epidemic strains C51, Type 4, and Type 27 have been made. It was found that Type 4 produced a more potent streptolysin O and a more active fibrinolysin than either C51 or Type 27.

The comparison of these cultures showed that the only notable difference between the Type 4 organisms, C51, and Type 27 was in the absence of a capsule and in the character of the colony. Although capsulated, mucoid streptococci are usually considered to be more virulent than matt strains, the upper respiratory infections associated with streptococcus Type 4 were more severe than those associated with streptococcus C51 and streptococcus Type 27. It is of interest, furthermore, that during the spring of 1939 when Type 4 was prevalent, 6 children had upper respiratory infections associated with capsulated mucoid streptococci of undetermined types. These 6 children also failed to develop rheumatic recurrences.

Coburn and Pauli (9) have described the isolation of Group A beta hemolytic streptococci which were ineffective in precipitating rheumatic recurrences in apparently susceptible rheumatic indi-

viduals. These authors were of the opinion that these strains differed from those associated with the subsequent development of rheumatic manifestations mainly in being poor toxin producers. In the experiments recorded above, no evidence was obtained to indicate that C51 and Type 27 produced particularly strong toxins.

The findings summarized in Table IV suggest that an unknown factor associated with streptococci which precipitates rheumatic fever may have been lacking in Type 4 organisms. Since both C51 and Type 27 appeared capable of precipitating rheumatic recurrences, it can be assumed that these two strains contained or carried the hypothetical special rheumatic factor. The failure of some children to develop rheumatic fever following infections with these two strains might be explained on the basis that these individuals were in a refractory state and therefore were insusceptible to rheumatic fever at that particular time.

It was thought of interest to compare the rheumatic histories of the 31 children who did not develop rheumatic recurrences following the streptococcus Type 27 pharyngitis with those of the 8 who did. Ten of the 39 children, who had upper respiratory infections associated with streptococcus Type 27 during the winter of 1939 to 1940, had had pharyngitis due to streptococcus Type 4 the previous year. Four children who had rheumatic recurrences following the Type 27 infections had escaped rheumatic sequelae following the Type 4 pharyngitis (Cases 1, 2, 4, and 7, Figure 1).

The rheumatic histories and cardiac findings before and after admission of the 39 children who had the Type 27 pharyngitis are presented in Figure 1.

According to Wilson (2), the rheumatic manifestation of greatest prognostic significance is carditis. Figure 1 shows that a large proportion, 6 out of 8, of the children who developed rheumatic recurrences following streptococcus Type 27 pharyngitis had had carditis. However, 14 of the 31 children who escaped rheumatic sequelae also had had definite cardiac involvement. Four children who were considered to be particularly vulnerable because their hearts were enlarged failed to develop rheumatic manifestations following the streptococcus Type 27 upper respiratory infection (Cases 23, 29, 35, 37, Figure 1). The average



TABLE III (continued)

Case number	Patient			Date	Clinical findings	Streptococcus Type 27, number of colonies	Latent period	Blood					
	Name	Age	Sex					Date	White blood cells	Sedimentation rate	Anti-streptolysin O titer		
8	T.L. 3508	years 13	F	April 19, 1940	Pharyngitis. No fever.	80%	days	March 2		3	80		
								April 9		1	60		
								April 15	6,800				
								April 20	8,500				
				May 9, 1940	Carditis (PR interval prolonged), erythema.			20	April 24	8,900	15		
									May 2	8,850	6	80	
									May 9	10,500	14	80	
									May 13	13,700	34	80	
								May 20	6,700	33	80		
								May 27		32	100		
								May 29	9,500				
								June 10	4,700	17	100		
								June 30		8	80		
								July 10	6,000				

Three children who still had some evidence of rheumatic activity when they were admitted in the fall developed exacerbations during the course of the winter. Twenty-three children who became carriers of streptococcus Type 27 showed no signs of rheumatic activity. None of the remaining 43 children in the institution developed rheumatic manifestations from November 1939 until July 1940.

#### *Comparison of the three epidemic strains isolated 1937 to 1940*

Since the effect of these three outbreaks of streptococcal upper respiratory infections varied greatly in regard to the incidence of rheumatic recurrences, it seemed of interest to compare the three epidemic strains. Therefore, streptococcus C51 (Case 2, Table I), streptococcus Type 4 (Lee) and Type 27 (Case 1, Table III), as well as one of the strains of undetermined type isolated in March 1939, 97T (Tudda), were compared as to capsule formation, colony form, mouse virulence, production of the type specific protein "M" and toxin production.

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viduals. These authors were of the opinion that these strains differed from those associated with the subsequent development of rheumatic manifestations mainly in being poor toxin producers. In the experiments recorded above, no evidence was obtained to indicate that C51 and Type 27 produced particularly strong toxins.

The findings summarized in Table IV suggest that an unknown factor associated with streptococci which precipitates rheumatic fever may have been lacking in Type 4 organisms. Since both C51 and Type 27 appeared capable of precipitating rheumatic recurrences, it can be assumed that these two strains contained or carried the hypothetical special rheumatic factor. The failure of some children to develop rheumatic fever following infections with these two strains might be explained on the basis that these individuals were in a refractory state and therefore were insusceptible to rheumatic fever at that particular time.

It was thought of interest to compare the rheumatic histories of the 31 children who did not develop rheumatic recurrences following the streptococcus Type 27 pharyngitis with those of the 8 who did. Ten of the 39 children, who had upper respiratory infections associated with streptococcus Type 27 during the winter of 1939 to 1940, had had pharyngitis due to streptococcus Type 4 the previous year. Four children who had rheumatic recurrences following the Type 27 infections had escaped rheumatic sequelae following the Type 4 pharyngitis (Cases 1, 2, 4, and 7, Figure 1).

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According to Wilson (2), the rheumatic manifestation of greatest prognostic significance is carditis. Figure 1 shows that a large proportion, 6 out of 8, of the children who developed rheumatic recurrences following streptococcus Type 27 pharyngitis had had carditis. However, 14 of the 31 children who escaped rheumatic sequelae also had had definite cardiac involvement. Four children who were considered to be particularly vulnerable because their hearts were enlarged failed to develop rheumatic manifestations following the streptococcus Type 27 upper respiratory infection (Cases 23, 29, 35, 37, Figure 1). The average

age of the 8 children who developed rheumatic recurrences was 11.6 years, and that of the 31 children who escaped was 9.8 years.

Coburn (10) is of the opinion that the type of immune response elicited by streptococcal pharyngitis in rheumatic subjects determines whether or not the rheumatic recurrences will develop. According to this observer, if the rise in antistreptolysin O titer is delayed rather than prompt, rheumatic sequelae are more likely to occur.

In 9 of the 14 rheumatic recurrences following streptococcal upper respiratory infections observed

by us, the rise in antistreptolysin O titer was not delayed. As already mentioned, 4 children who developed rheumatic manifestations following Type 27 streptococcus pharyngitis had had the non-effective Type 4 streptococcus upper respiratory infection during the spring of 1939. The immune response of these 4 patients to these two infections was identical. The rise in the antistreptolysin O titer of 2 of these children, both to the streptococcus Type 4 and Type 27, was marked and prompt. One child failed to show a rise either to the Type 4 or to the Type 27, and in the other child the rise in both instances was doubtful.

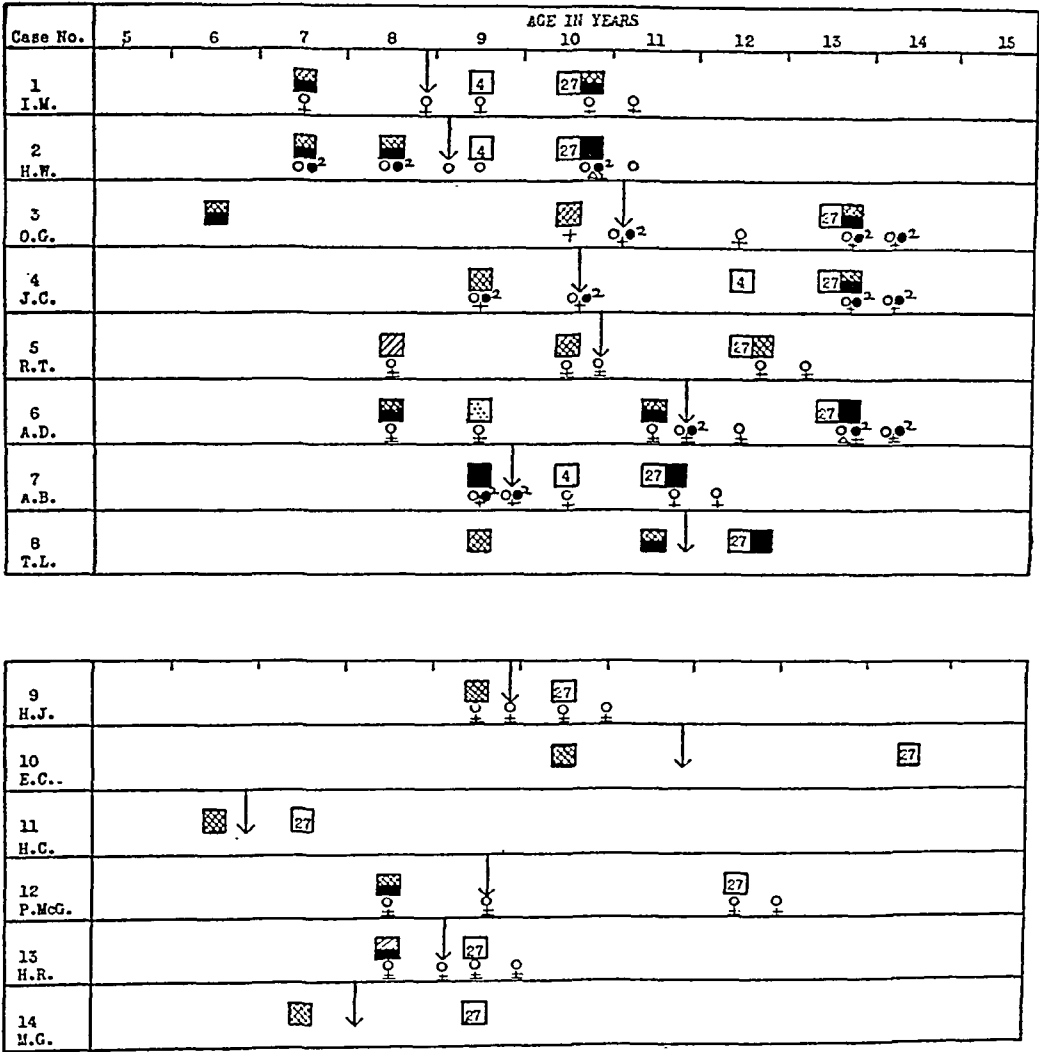


FIG. 1. RHEUMATIC HISTORIES OF 39 PATIENTS WHO DEVELOPED STREPTOCOCCUS TYPE 27 PHARYNGITIS

Cases 1 to 8 represent the rheumatic histories of the 8 patients who developed rheumatic recurrences. Cases 9 to 14 represent the rheumatic histories of the 6 patients who showed a rise in antistreptolysin O titer without developing rheumatic sequelae. Cases 15 to 39 showed no rise in antistreptolysin O titer and no rheumatic manifestations.

\* The symbols used in this Figure were devised by the New York Heart Association.

# STREPTOCOCCAL INFECTIONS IN RHEUMATIC FEVER

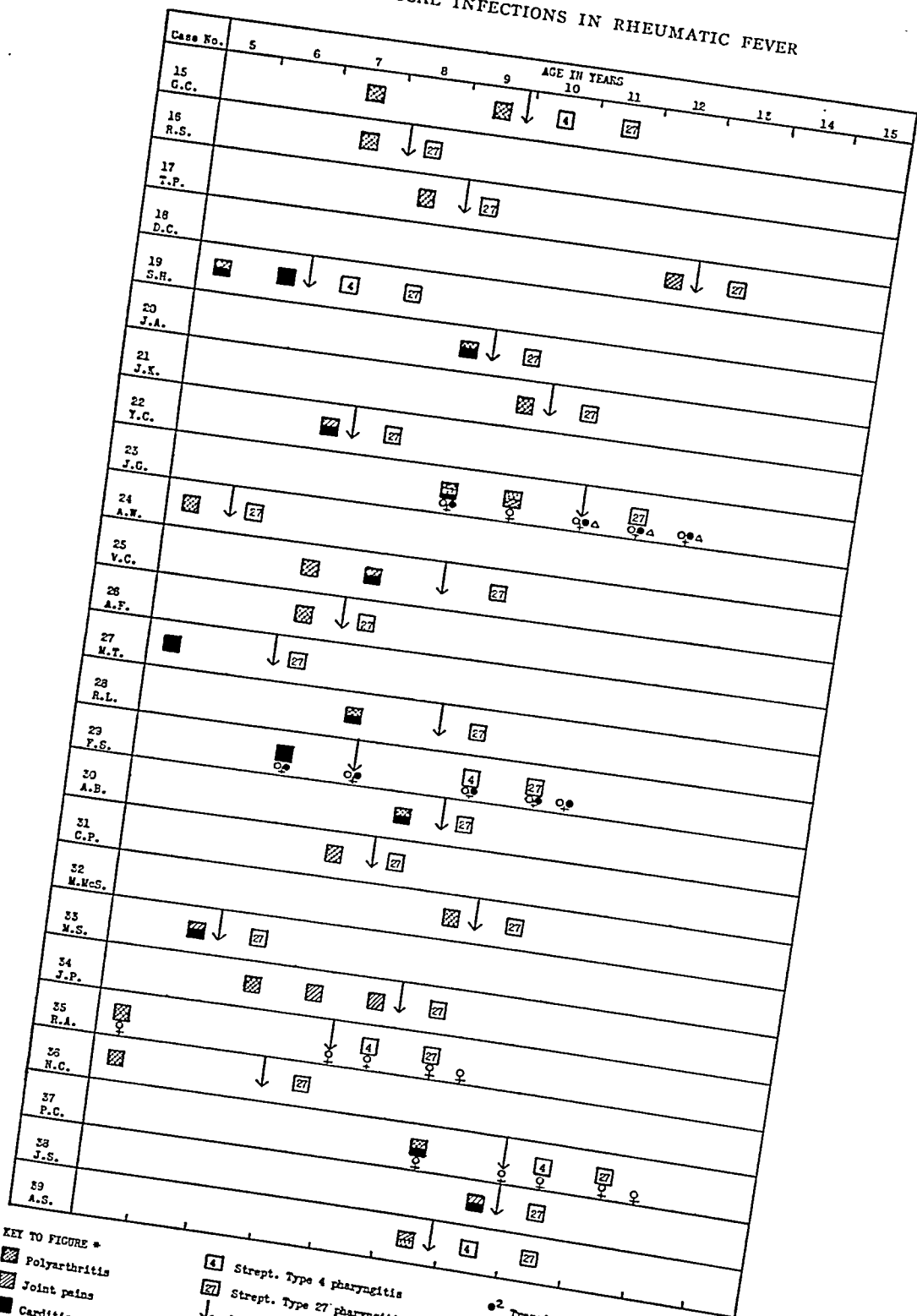


FIG. 1

## SOURCE OF THE EPIDEMIC STRAINS

In view of the fact that outbreaks of streptococcal upper respiratory infections are considered likely to precipitate rheumatic recurrences, it is the policy of some sanatoria and convalescent homes for rheumatic children to exclude children who are streptococcus carriers. It therefore was thought of interest to trace the probable source of each of the epidemic strains and also report the findings in regard to Group A beta hemolytic streptococcus carriers during each of the three winters.

Children who were found to be streptococcus carriers on admission usually carried small numbers of organisms and therefore were not excluded. Only one boy who had chronic sinusitis with marked purulent nasal discharge, which contained Group A beta hemolytic streptococci in almost pure culture, was sent home immediately.

*1937 to 1938*

The epidemic strain of this year, streptococcus C51, was introduced by a carrier admitted in May 1937 and discharged in October of that year. During the summer and fall of 1937, 5 other children became carriers of this strain. The first case of streptococcus C51 pharyngitis appeared at the end of December 1937.

*Carriers.* From December 1, 1937 until June 1, 1938, 17 children were carriers of Group A beta hemolytic streptococci other than the epidemic strain C51. No cases of pharyngitis developed which could be attributed to infection with any of these 17 strains.

*1938 to 1939*

The epidemic strain of this year, streptococcus Type 4, was first isolated from a child with acute pharyngitis. Since streptococcus Type 4 had not been present before, it was thought probable that this child had contracted the upper respiratory infection from her parents who had visited her two days before she became ill. The source of streptococcus 97T, which was considered the cause of the other 6 cases of pharyngitis occurring during this year, was not determined.

*Carriers.* From December 1, 1938 until June 1, 1939, 19 children were carriers of Group A beta

hemolytic streptococci other than the epidemic strain, streptococcus Type 4. Among these 19 strains, 6 were found to be Types 5, 12, 17, 18, 19, and 29, respectively. One child who had had streptococcus C51 pharyngitis during March 1938 continued to carry this organism until June 14, 1939. The types of the remaining 12 carrier strains were not determined. No cases of pharyngitis developed which could be attributed to infection with any of these 19 strains.

*1939 to 1940*

The epidemic strain of this year, streptococcus Type 27, was introduced by a child who had a tonsillectomy done in another hospital. She was readmitted two days after the operation. She had fever for five days after her return, and during that time throat cultures showed large numbers of streptococcus Type 27.

A second strain of undetermined type, designated as R31, was also prevalent during this year. This organism was introduced in June 1939. A large number of children, a total of 22, gradually became carriers of this strain. Only one child developed a mild pharyngitis due to this organism. After a latent period of fourteen days, this patient showed low-grade symptoms of rheumatic activity.

*Carriers.* From December 1, 1939 until June 1, 1940, 11 children were carriers of Group A beta hemolytic streptococci other than streptococcus R31 and the epidemic strain, streptococcus Type 27. Four children who were in the institution during the spring of 1939 carried the epidemic strain of that year, streptococcus Type 4, during the winter of 1939 to 1940.

Of the 11 carrier strains 6 were found to be Types 2, 4, 6, 12, 17, and 29, respectively. The types of the other 5 carrier strains were not determined. No cases of pharyngitis developed which could be attributed to infection with any of these 11 strains.

## DISCUSSION

Three outbreaks of streptococcal upper respiratory infections occurring during three successive winters in a sanatorium for rheumatic children are reported. Each epidemic was associated with Group A beta hemolytic streptococci of a single

type, but a different type was prevalent in each outbreak. The incidence of rheumatic recurrences following these three waves of streptococcal pharyngitis varied greatly. In the first outbreak, following a latent period, half the children showed signs of rheumatic activity; in the second, all the children escaped rheumatic sequelae; and in the third, a relatively small proportion developed rheumatic recurrences. A comparison of the two types of streptococci associated with recurrences (*i.e.* "effective" strains) and the "non-effective" strain failed to show any significant differences.

At the present time, there is, in our opinion, no adequate explanation of why rheumatic manifestations sometimes follow in the wake of streptococcal upper respiratory infections and sometimes do not. It seems possible that either the "non-effective" strains of Group A beta hemolytic streptococci lack some unknown, hypothetical factor necessary for the precipitation of rheumatic recurrences, or that the vulnerability of rheumatic subjects to the effect of streptococcal upper respiratory infections varies greatly from year to year.

Following infection with the two "effective" strains of streptococci, rheumatic recurrences developed sporadically over a period of months. It therefore must be assumed that these organisms carried the hypothetical rheumatic factor throughout the epidemics. However, a large number of children who had streptococcal pharyngitis due to these strains failed to develop demonstrable signs of rheumatic activity, suggesting that these individuals may have been in a refractory state. A comparison of the rheumatic histories of the children who did and those who did not develop rheumatic recurrences showed no striking differences.

An analysis of the previous rheumatic attacks of the 14 children who developed rheumatic recurrences following streptococcal upper respiratory infections showed that the rheumatic manifestations in each patient tended to follow a certain pattern characteristic of the particular individual. Mild and severe rheumatic manifestations followed infection with the same strain of Group A beta hemolytic streptococci.

The effect of streptococcal upper respiratory infections in precipitating rheumatic sequelae has usually been studied in rheumatic patients with marked cardiac damage who are known to be sub-

ject to frequent rheumatic recurrences. In this study, children in the early stages of rheumatic fever were selected because it is of primary importance to determine what factors precipitate recurrences before marked cardiac damage has developed. It has been found (11) that in the majority of cases severe cardiac involvement occurs within five years after the initial attack of polyarthritis or carditis. At the present time, there is no way of predicting the subsequent course of a child shortly after his first attack of polyarthritis or carditis. It is therefore of interest that 31 children (Cases 9 to 39, Figure 1) who contracted the "effective" streptococcus Type 27 pharyngitis within five years following their first attack of polyarthritis or carditis escaped rheumatic sequelae.

During 1937 to 1938 the probable source of the epidemic strain was a patient who was a streptococcus carrier. During 1938 to 1939 and 1939 to 1940 the probable sources of the epidemic strains were 2 children who developed acute upper respiratory infections as the result of outside contacts. During all three years, a fairly large proportion of the children carried Group A beta hemolytic streptococci of various types which, in most instances, did not spread or cause pharyngitis. It is noteworthy that some of these carrier strains had capsules and formed mucoid colonies. It is our impression that cases of upper respiratory infections due to types of streptococci not previously present in the community are the most likely sources of epidemic strains. Occasionally, a carrier strain may spread slowly from child to child and after several passages produce clinical symptoms of upper respiratory disease. However, at the present time, it is impossible to predict which carrier strain will behave in this way.

In view of these findings, it does not seem to us that the exclusion of rheumatic children from sanatoria or convalescent homes because they are carriers of small or moderate numbers of Group A beta hemolytic streptococci is warranted. One or two throat cultures taken before admission are not adequate to exclude carriers. Children who are carriers may have negative throat cultures for several weeks, or months, and then again show fairly large numbers of streptococci of the same type.

A large proportion of children who have had

streptococcal pharyngitis continue to carry streptococci for months after recovery. It has been found by several observers (12), as well as ourselves, that although sulphanilamide may reduce the number of streptococci carried temporarily, this drug does not clear up carriers permanently. At the present time, no effective way of clearing up streptococcus carriers is known and it is not feasible to isolate children for months at a time. It therefore is impossible to eliminate Group A beta hemolytic streptococci even in a relatively isolated group of children.

As far as explaining the rôle of Group A beta hemolytic streptococci in the etiology of rheumatic fever is concerned, our observations leave many questions unanswered. Although the total number of rheumatic recurrences, namely fourteen, observed during the three winters among more than 300 rheumatic children is small, it is striking that all of these followed in the wake of streptococcal pharyngitis. No child who escaped streptococcal upper respiratory infections developed rheumatic manifestations. On the other hand, many children who contracted infections with the "effective" strains of streptococci remained well.

At the present time, the situation in regard to rheumatic fever is analogous to that which prevailed in regard to scarlet fever before the discovery of erythrogenic toxin and the Dick test. We now know that toxin-producing strains of streptococci are likely to produce scarlet fever in Dick-positive individuals. In rheumatic fever, we are unable to ascertain the state of susceptibility of the patient or what special property or factor makes certain strains of streptococci "effective." It seems possible that fluctuations in the resistance of rheumatic individuals from year to year may be more important in precipitating rheumatic recurrences than qualitative or quantitative differences between various strains of streptococci.

It is undoubtedly important to continue the study of the relationship of streptococci to the etiology of rheumatic fever. However, another approach to the problem should also be borne in mind. It seems possible that the streptococcus may be merely an indicator which accompanies the invasion or reactivation of a hypothetical rheumatic agent. The entrance or increased activity of this agent may enhance the infectivity of streptococci, just as the multiplication of herpes sim-

plex is facilitated by certain diseases which apparently lower the resistance of the patient. If this hypothesis is true, the failure of certain children to develop rheumatic sequelae following pharyngitis due to "effective" strains of streptococci might be explained on the basis that re-infection with exogenous rheumatic "virus" or reactivation of endogenous rheumatic "virus" had not taken place at that particular time. The latent period observed following "effective" streptococcal upper respiratory infections might represent the interval after the new attack or reactivation necessary for the rheumatic agent to multiply sufficiently to produce demonstrable symptoms. Strains of streptococci would then be "effective" or "non-effective" depending on whether or not they were coincident with a fresh infection or a reactivation of the rheumatic agent. The proof of this hypothesis depends on the isolation of a rheumatic "virus."

#### CONCLUSIONS

1. The effect of three outbreaks of streptococcal upper respiratory infections during three successive winters in a colony of rheumatic children are described. Each of these outbreaks was due to infections with a single type of Group A beta hemolytic streptococci, but during each epidemic a different type was prevalent.

2. The incidence of rheumatic recurrences following these streptococcal upper respiratory infections varied greatly—from none to a large proportion of the cases.

3. A comparison of the epidemic strains failed to reveal any significant differences which might account for the variations in the incidence of rheumatic recurrences.

4. A comparison of the rheumatic histories of children who escaped and of those who developed rheumatic recurrences following pharyngitis due to "effective" strains of streptococci likewise did not show any striking differences. Our findings suggest that the vulnerability of the rheumatic subject to the effect of streptococcal upper respiratory infections is variable, and depends on factors which at the present time are not understood.

5. No rheumatic recurrences were observed in children who escaped streptococcal upper respiratory infections during the three-year period.

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# AN EPIDEMIC OF INFLUENZA B OCCURRING IN A GROUP OF RHEUMATIC CHILDREN CONCURRENT WITH AN OUTBREAK OF STREPTOCOCCAL PHARYNGITIS: CLINICAL AND EPIDEMIOLOGICAL OBSERVATIONS<sup>1</sup>

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During the past three years, 1937 to 1940, the effect of upper respiratory infections on the reactivation of the rheumatic process has been studied at Irvington House, a sanatorium for rheumatic children. During each winter, in addition to sporadic outbreaks of the "common cold," a series of cases of streptococcal pharyngitis developed (1). In February 1940, a third type of upper respiratory infection, epidemic influenza, was observed for the first time. Fifty cases of influenza developed in rapid succession. Dr. Thomas Francis, Jr. of the New York University College of Medicine succeeded in isolating an influenza virus of a hitherto undescribed type, designated as B, from the throat washings of one of the cases (2).

Since this is the first epidemic proven to be due to influenza virus B, the clinical and epidemiological findings were considered to be of interest. Furthermore, streptococcal pharyngitis was prevalent in the community, and the opportunity for studying the effect of these two types of upper respiratory infections on each other was excellent.

In 1935, Coburn and Pauli (3) reported an epidemic of "influenza" in a group of rheumatic children which appeared to facilitate the spread of Group A beta hemolytic streptococcus infections. However, it seems possible that this outbreak of upper respiratory disease was not epidemic influenza, since the leukocyte counts of the patients tended to be elevated rather than depressed (4). A ferret pathogenic virus was not isolated.

Since the outbreak of streptococcal pharyngitis began before the epidemic of Influenza B, the streptococcal upper respiratory infections will be described first. The type of community and routine procedures are described in the preceding paper.

## 1. Outbreak of streptococcal pharyngitis: November 2, 1939 to April 19, 1940

On November 2, 1939, a girl who had had a tonsillectomy in another hospital was readmitted. She had fever for six days after her return, and throat cultures, which had been negative before operation, showed large numbers of Group A beta hemolytic streptococci, Type 27. During November, 4 other girls developed pharyngitis, and their throat cultures were positive for streptococcus Type 27. The infection spread slowly, with 8 cases in December, and reached its peak with 16 cases in January. Three new cases developed in the first half of February, 6 in March, and 1 in April. During the six months when the streptococcal pharyngitis was prevalent, 23 children became carriers of streptococcus Type 27 without developing clinical symptoms.

*Clinical findings in 39 children with streptococcus Type 27 pharyngitis.* With a few exceptions, all the children complained of sore throat. Thirty-three of the 39 cases had fever ranging from 104.2 to 100°, which subsided after three days in all but 4 instances. The pharynx, in most of the patients, appeared inflamed and slightly edematous. Exudate was noted only in children who had tonsils. Only 2 patients had coryza. Three children developed cervical adenitis with a secondary rise in temperature seven, eight and fifteen days, respectively, after the initial infection. No other complications were observed.

After a latent period varying from eleven to twenty-two days, 8 of these 39 children developed rheumatic recurrences (1).

*Laboratory data, streptococcus Type 27 pharyngitis.* A well-marked leukocytosis with an increase in the percentage of polymorphonuclear

<sup>1</sup> This work was aided by a grant from The Commonwealth Fund.

cells was the characteristic finding in most of the cases. The total leukocyte counts ranged from 20,700 to 7,400, with an average count of 13,100. Following the pharyngitis, 28 children showed a well-defined rise in the erythrocyte sedimentation rate and 10 children a rise in antistreptolysin O titer.

In February, when the incidence of streptococcal pharyngitis appeared to be declining a sudden outbreak of an acute, febrile disease clinically distinct from the streptococcal infections appeared.

## 2. Outbreak of Influenza B: February 16 to March 18, 1940

On February 16, 1940, a girl developed fever of 100.6° for one day. She had no complaints and no signs of upper respiratory infection. Her leukocyte count was found to be 5,700. Throat cultures were negative for Group A beta hemolytic streptococci. Three days later a boy complained of malaise and his temperature was 102.4 to 100° for five days. His leukocyte count was 8,600. On the following day, 3 more children (2 boys and 1 girl) had fever. None of these children complained of "sore throat" or had symptoms suggesting the common cold. The leukocyte counts were uniformly low, and throat cultures were negative for Group A beta hemolytic streptococci. Further cases developed in rapid succession until a total of 50 cases was reached within a period of thirty-one days. The percentage of susceptible individuals, 46 per cent of 108 children, was high. The percentage of girls affected, namely 53 per cent (35 of the 66 girls), was greater than the percentage of boys, 35 per cent (15 of the 42 boys). All the children who contracted epidemic influenza had been in the institution two and one-half months or longer, and were considered to be in good general condition. Half of the 108 children were receiving large doses of vitamins A, B complex, C and D, in addition to the regular, well-balanced diet (5). However, the incidence of epidemic influenza in children receiving the same diet without additional vitamins, and those receiving an excess of vitamins, was the same. None of the 40 adults who lived in the institution contracted the disease.

*Clinical findings in 50 children with Influenza B.* The onset was abrupt, with an elevation of

temperature. The most common complaints were headache, drowsiness and slight malaise. Thirteen children had no complaints at any time. Mild coryza was noted in 10 of the 50 children on the second or third day of illness. Four children developed a slight cough. Myalgia was present in only 4 instances. The average duration of fever was four days with a maximum of 104° F. A secondary rise in temperature after one to three days occurred in 7 children. Gastro-intestinal symptoms were not observed. In the majority of children, the symptoms were mild. Physical examination revealed no abnormal findings. The pharynx did not appear inflamed and the lungs were clear. All the children recovered rapidly and no complications of any kind developed. The postinfluenzal lassitude, so commonly seen in adults, was absent. No rheumatic recurrences followed in the wake of the influenzal outbreak.

The contrast in the clinical findings of these two types of upper respiratory infection, streptococcal pharyngitis and Influenza B, was striking and is presented in Table I.

TABLE I  
*Comparison of complaints and symptoms in 39 cases of streptococcus Type 27 pharyngitis and 50 cases of Influenza B*

	Streptococcal pharyngitis	Influenza B
Complaints:		
"Sore throat".....	35 patients	0
"Headache".....	3 patients	16 patients
Drowsiness.....	0	13 patients
Malaise.....	1 patient	10 patients
Myalgia.....	0	4 patients
None.....	0	13 patients
Symptoms:		
Onset.....	gradual	abrupt
Duration of fever (average).....	2 days	4 days
Pharynx.....	inflamed, edematous	normal
Cervical adenitis.....	3 patients	none
Coryza.....	2 patients	10 patients (mild)
Cough.....	0	4 patients (slight)

*Laboratory data, Influenza B.* In contrast to the leukocytosis observed in the patients with streptococcal pharyngitis, a relative leukopenia with no increase in the percentage of polymorphonuclear cells was the characteristic finding in cases of the virus infection. The average leukocyte count was 5,600. The range of the leukocyte counts found in these two types of acute respiratory infections is presented in Figure 1.

The percentage of polymorphonuclear cells in the two diseases also showed marked differences

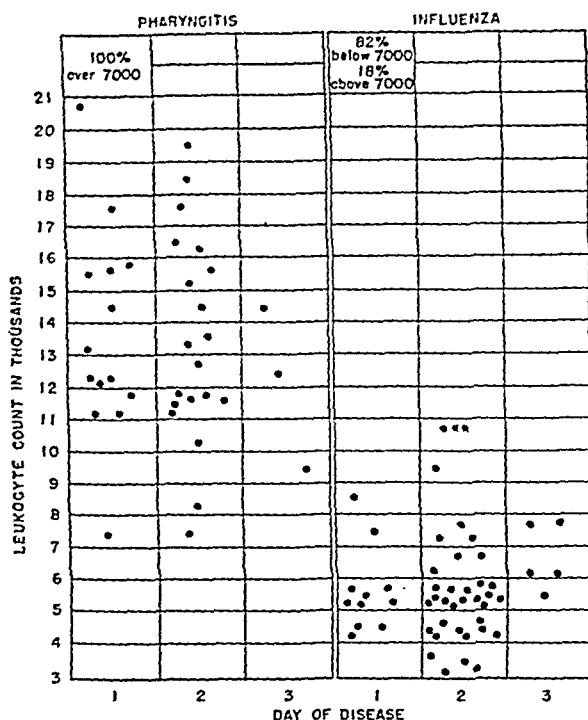


FIG. 1. LEUKOCYTE COUNT IN 50 CASES OF INFLUENZA AND 37 CASES OF STREPTOCOCCUS TYPE 27 PHARYNGITIS.\*

\* Leukocyte counts were not obtained on 2 of the patients with streptococcal pharyngitis.

\*\* This child had had infectious mononucleosis. With the onset of Influenza B, her leukocyte count dropped from 31,000 to 10,400 in two days.

and is presented in Table II. Only 13 of the 50 children showed a slight transient rise in the erythrocyte sedimentation rate. With the exception of known carriers, throat cultures were uniformly negative for Group A beta hemolytic strep-

TABLE II

*Differences in the polymorphonuclear response to streptococcal pharyngitis and Influenza B*

Polymorphonuclear cells	Streptococcal pharyngitis		Influenza B	
	Number of cases	Per cent	Number of cases	Per cent
90, or above....	1	2.6		
80 to 90.....	8	21.6		
70 to 80.....	15	40.5	4	8
60 to 70.....	10	27.2	19	38
60, or below....	3	8.1	27	54
Total.....	37	100.0	50	100

tococci. No rise in antistreptolysin O titer occurred.

*Epidemiological data.* Not only the clinical and laboratory but also the epidemiological picture of the two diseases varied greatly. In contrast to the streptococcal epidemic, the onset and termination of the influenza outbreak were abrupt, a total of 50 cases developing within a period of thirty-one days. The difference in the mode of spread of the streptococcal pharyngitis and the Influenza B is presented in Figure 2.

### 3. The relationship of the influenza epidemic to the outbreak of streptococcal pharyngitis

Thirty-two cases of streptococcus Type 27 pharyngitis had occurred in the community before the beginning of the influenza epidemic on February 16, 1940.

During the first twelve days of the influenza outbreak, when 25 cases of the virus infection developed, no new cases of streptococcal pharyngitis were observed. During the second half of the influenza epidemic, when 25 more cases of influenza appeared, only 2 children who escaped the virus infection developed streptococcal pharyngitis. From March 19 to April 19, when the influenza outbreak was over, 5 new cases of streptococcus Type 27 pharyngitis developed. Three of these 5 children had escaped Influenza B. Only 2 patients who had had influenza subsequently contracted the streptococcus Type 27 pharyngitis; in one case, the interval between the virus and streptococcal infections was two weeks, and in the other, four weeks. The clinical findings in these 7 children were similar to the 32 cases of streptococcal pharyngitis which occurred before the influenza outbreak. No increase in severity of the symptoms was observed and no complications developed.

Of the 50 children who contracted Influenza B, 21 had had streptococcus Type 27 pharyngitis. Twelve of these 21 patients were still carrying streptococcus Type 27 during the influenza epidemic. Nine children who had become carriers of streptococcus Type 27 developed influenza. No increase in the number of streptococci carried by these children was detected. Two children who developed influenza were carriers of Group A beta hemolytic streptococci of undetermined types. No evidence of spread of these organisms

was observed. The data in regard to previous and subsequent infection with streptococcus Type 27 and the number of streptococcus carriers in the group of 50 patients with Influenza B are presented in Figure 3.

#### DISCUSSION

In the past few years, several outbreaks of acute respiratory disease clinically indistinguishable from epidemic influenza have been reported in which attempts to isolate an etiological agent were unsuccessful and no relationship to known strains of influenza virus could be established (6). In a recent article (7), it was suggested that the term Influenza A should be used to designate infections caused by any of the known strains of influenza virus, and that new types of virus could then be named Influenza B, C, et cetera.

The outbreak of epidemic influenza occurring at Irvington House, described in this paper, is of special interest because a new virus serologically distinct from Influenza A was isolated by Francis (2). Furthermore, by immunological studies this

author showed that the same or a very closely related etiological agent was also responsible for other epidemics occurring in widely separated parts of the United States of America. In accordance with the terminology recently suggested (7), Francis has designated this virus Influenza B (2).

The symptoms caused by Influenza B closely resembled those associated with Influenza A. The disease was mild, with constitutional rather than catarrhal symptoms predominating. The leukocyte counts in the 50 Influenza B patients consistently showed a relative leukopenia.

Cases of pharyngitis due to a single type of Group A beta hemolytic streptococci, Type 27, were prevalent in the community before the outbreak of Influenza B. No evidence was obtained to suggest that Influenza B facilitated the spread of Group A beta hemolytic streptococci.

The epidemiology, as well as the clinical and laboratory findings in these two types of acute respiratory disease, differed greatly. The explosive character of the Influenza B outbreak indicated that all the susceptible individuals in the

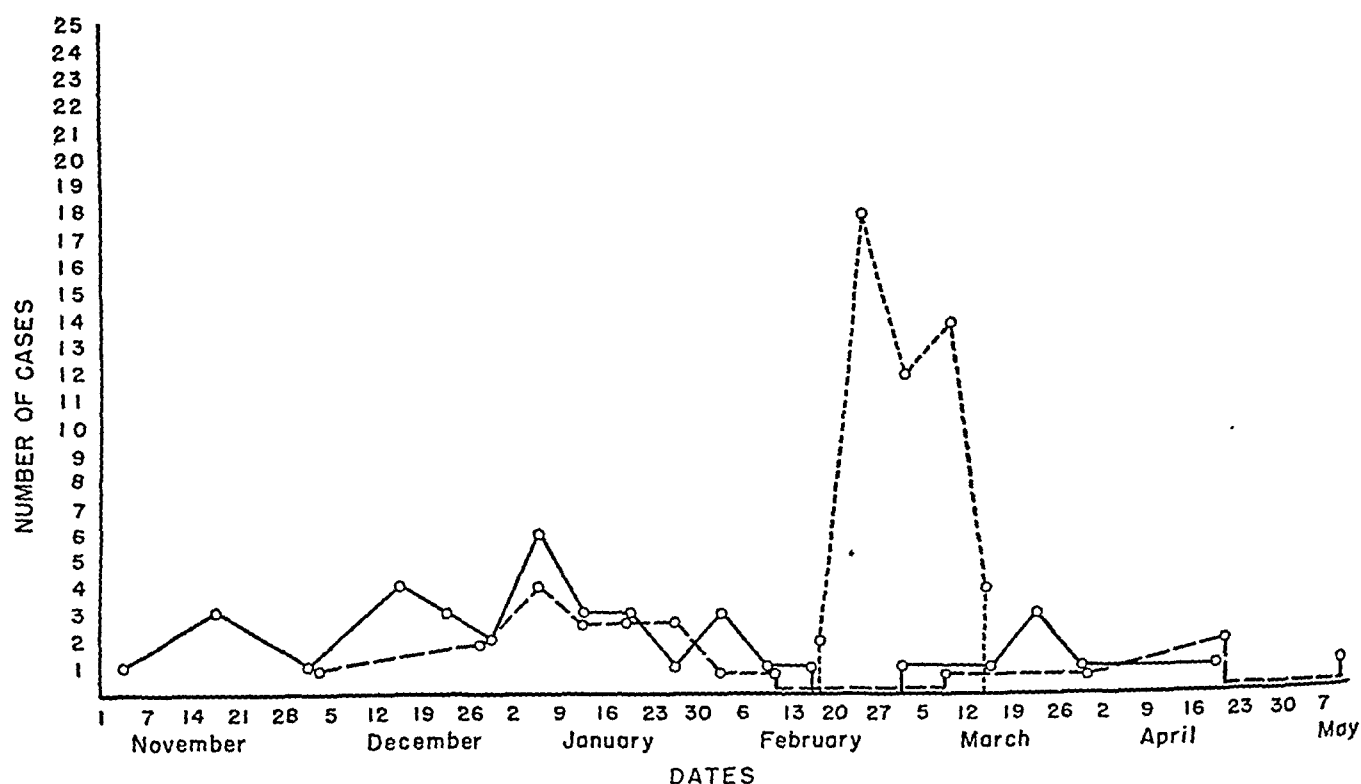


FIG. 2. WEEKLY INCIDENCE OF STREPTOCOCCAL PHARYNGITIS AND EPIDEMIC INFLUENZA FROM NOVEMBER 1939 TO JUNE 1940

○—— Pharyngitis, Type 27  
 ○--- Carriers, Type 27  
 ○---- Epidemic Influenza

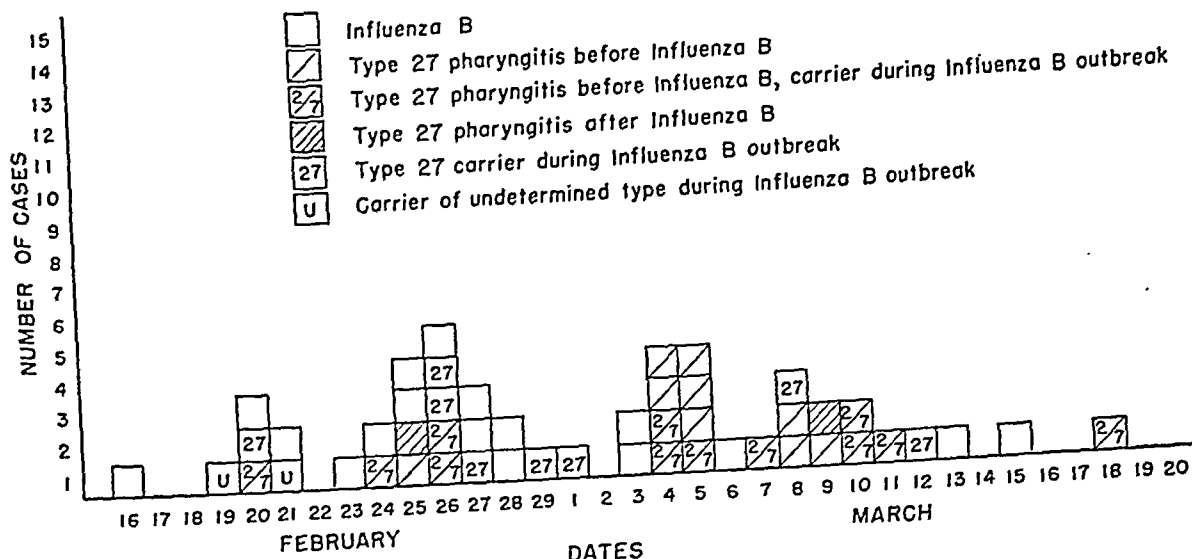


FIG. 3. DATA OF PREVIOUS AND SUBSEQUENT INFECTION WITH STREPTOCOCCUS TYPE 27 AND THE NUMBER OF STREPTOCOCCUS CARRIERS IN THE GROUP OF 50 PATIENTS WITH INFLUENZA B

community contracted the infection in a relatively short time. Susceptibility to the streptococcus Type 27 pharyngitis was much less marked, since new cases developed over a period of six months. Furthermore, the susceptibility of the same child seemed to vary from time to time because, in a few instances, in spite of intimate exposure to an acute case of the streptococcal infection, children failed to develop pharyngitis, only to contract the disease several weeks later.

The most striking difference in the clinical picture was the absence of signs and symptoms referable to the upper respiratory tract in most of the cases of Influenza B. In the streptococcal infections nearly all the patients complained of "sore throat," and definite inflammation of the pharynx was usually apparent. The laboratory finding of the greatest value in establishing the diagnosis of Influenza B was the leukocyte count. By means of the leukocyte count, it was usually possible to differentiate cases of the virus infection from cases of streptococcus Type 27 pharyngitis on the first day of disease. A relative leukopenia was found in all the patients with Influenza B and a definite leukocytosis in the majority of patients with the streptococcal infection. Following a latent period 8, or 20 per cent, of the 39 patients who had the streptococcus Type 27 pharyngitis developed rheumatic recurrences, whereas no in-

stance of a reactivation of the rheumatic process was observed following Influenza B.

#### CONCLUSIONS

1. An outbreak of influenza due to a recently described influenza virus, Influenza B (2), in a relatively isolated group of rheumatic children has been described.
2. The clinical symptoms were mild and remained remarkably uniform throughout the epidemic. No complications of any kind developed.
3. The characteristic laboratory finding was a relative leukopenia.
4. No evidence was obtained to suggest that the virulence of a Group A beta hemolytic streptococcus of proven pathogenicity was increased by this strain of influenza virus.
5. Rheumatic recurrences were not precipitated by the influenza outbreak.

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# STUDIES OF CALCIUM AND PHOSPHORUS METABOLISM. XVI. THE INFLUENCE OF THE PITUITARY GLAND<sup>1</sup>

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The literature pertaining to the effects of the anterior pituitary gland on calcium and phosphorus metabolism, well summarized by Zwarenstein (1) and Houssay (2), is meager and the results reported are contradictory. This is due in part to the fact that many of the previous workers employed inert anterior pituitary gland preparations.

In 1934 Hertz and Kranes (3) and Anselmino, Hoffmann and Herold (4) found that injections of an alkaline extract of the anterior pituitary gland into rabbits and rats caused hypertrophy of their parathyroid glands. Marenzi and Gerschman (5), working with dogs, were unable to demonstrate such an effect regularly.

Alterations in the blood calcium have rarely been observed following either hypophysectomy or the administration of anterior pituitary gland extracts. Hogben (6) did report the occurrence of a low blood calcium in the toad (*Xenopus Laevi*) following removal of the anterior pituitary and the pars intermedia. He further stated that injections of pituitary extracts caused a fall in the blood calcium. These observations have been confirmed by Shapiro and Zwarenstein (7).

The evidence suggesting that the pituitary influences the parathyroid glands and calcium and phosphorus metabolism in higher animals is even more conflicting. Gerschman and Marenzi (8), working in Houssay's laboratory, found no change in the blood calcium of hypophysectomized dogs but did observe an elevation following the administration of an alkaline anterior pituitary extract, a finding previously noted in one dog by Thompson and Cushing (9). Subsequently, Marenzi and Gerschman (10) reported that such extracts were without effect on the blood calcium of four thyroparathyroidectomized dogs. Because of the

extreme difficulty in maintaining such experimental animals in a constant state, too much significance cannot be attached to these results. Friedgood (11) and Friedgood and McLean (12) noted increased blood calcium levels in rats and guinea pigs when alkaline anterior pituitary extracts were given. A tendency to a negative calcium balance in hypophysectomized rats, counteracted by injections of growth-promoting pituitary extracts, was observed by Pugsley and Anderson (13).

The literature may be summarized as follows: Injections of alkaline anterior pituitary extracts produce hypertrophy of the parathyroid glands and may cause elevation of the blood calcium level. It has not been definitely proved that this latter effect is lost in the absence of the thyroid and parathyroid glands. Hypophysectomy does not alter the blood calcium level of animals other than the toad.

The evidence favoring a relationship of the pituitary to the parathyroid glands and calcium and phosphorus metabolism in man consists of histological material collected by Cushing and Davidoff (14) and metabolism studies on two acromegalic patients (15, 16). Scriver and Bryan (15) studied a case of acromegaly with marked osteoporosis. Although they demonstrated the existence of a negative calcium balance, they presented no data supporting the view that hyperfunction of the thyroid and parathyroid glands played any rôle in the production of this metabolic alteration. The case studied by Langeron *et al.* (16) showed a reversal to a positive calcium balance following roentgen ray therapy to the pituitary gland. The metabolic regime which they employed was not described, the dietary regime was most haphazard, and only two one-day metabolic studies were made.

The purpose of this report is to present the calcium, phosphorus and nitrogen metabolism studies of five patients suffering from varying

<sup>1</sup> The expenses of this investigation were aided in part by a grant from the Commonwealth Fund.



TABLE I  
*Calcium, phosphorus and nitrogen metabolism in acromegaly*

Case number	Duration of disease	Degree of hypoparathyroidism present at time of study	Basal metabolic rate	Sugar tolerance curve	Serum calcium	Serum phosphorus	Number of 3-day metabolic periods averaged	Calcium				Phosphorus				Nitrogen			
								Urine	Feces	Total	Intake	Balance	Urine	Feces	Total	Intake	Balance	Urine	Total
								grams per 3-day period				grams per 3-day period				grams per 3-day period			
I Mrs. M.T.B. Age 33	6+	Moderate to severe	+27	Abnormal	10.4	4.7	4	1.10	0.67	1.77	0.30	-1.47	2.73	0.67	3.40	2.14	-1.36	30.82	33.65
II Mr. M.K. Age 30	4+	Slight to moderate	-22	Normal	10.2	5.2	3	0.89	0.29	1.18	0.35	-0.83	1.86	0.94	2.80	2.73	-0.07	36.50	40.48
III* Mr. R.J. Age 56	10+	Slight to moderate	+7 to +26	Abnormal	10.7	4.7	4	0.57	0.34	0.91	0.30	-0.61	1.51	0.53	2.04	1.78	-0.26	22.39	24.84
IV Mrs. M.D. Age 34	8+	Slight	+17	Abnormal	9.5	5.8	3	0.54	0.41	0.95	0.32	-0.63	2.16	0.47	2.63	2.10	-0.53	28.23	31.28
V Mrs. R.M. Age 46	6+	Very slight evidence of activity	-24	Not done	9.6	4.4	3	0.17	0.52	0.69	0.39	-0.30	1.27	0.71	1.98	2.05	+0.07	25.60	28.56

\* This patient was referred to us from the Peter Bent Brigham Hospital by Dr. Merrill Sosman.

grades of uncomplicated acromegaly, to discuss the alterations observed and to comment upon the effects of roentgen ray therapy.

### METHODS

The metabolic methods of ward management and analyses of blood and excreta were the same as we have employed previously (17). The patients were maintained on a constant low calcium, neutral diet similar to that used in later studies (19, 20), thus affording a satisfactory basis of comparison with our normal control subjects who were individuals between 19 and 60 years of age. The patients were all conscientious individuals, well suited for metabolic investigations.

### RESULTS

Four patients with varying grades of acromegaly all showed a calcium excretion well above that found in normal individuals on the same dietary intake. Case V, who was believed to have only very slight evidence of increased activity of the pituitary, showed a normal excretion (Table I). The average calcium excretion per 3-day period, shown in Table II, is seen to be approximately twice that of the control subjects. It is further noted that the excess calcium appears in the urine. Expressed as milligrams of calcium excreted per kilogram body weight, the acromegalics excreted 19 mgm. per kgm., as compared to 12.7 mgm. per kgm. for the normal individuals. The highest value, 27.4 mgm. per kgm., was seen in Case I. As will be seen from Table I, the calcium excretion was higher in the patients who clinically had the more active disease.

TABLE II

*Average calcium excretion per 3-day period on a low calcium neutral diet*

	Number of patients	Urine	Feces	Total
		grams	grams	grams
Controls.....	9	0.19	0.39	0.58
Acromegalics.....	5	0.65	0.45	1.10
Excluding Case V.	4	0.78	0.43	1.21

Two of the acromegalics (Case I and Case III) were studied before and after roentgen ray therapy to the pituitary gland. Table III shows that in Case I both the calcium and phosphorus excre-

TABLE III

*Average calcium and phosphorus excretion per 3-day period before and after roentgen ray therapy*

	Number of 3-day metabolic periods averaged	Calcium			Phosphorus			Comment
		Urine	Feces	Total	Urine	Feces	Total	
		grams	grams	grams	grams	grams	grams	
Case I Before therapy	4	1.10	0.67	1.77	2.73	0.67	3.40	The dietary intake for each patient was the same before and after treatment (See Table I)
After therapy	3	0.69	0.54	1.23	2.13	0.69	2.03	
Case III Before therapy	4	0.57	0.34	0.91	1.51	0.63	2.23	
After therapy	3	0.77	0.30	1.07	1.65	0.67	3.40	

tions were reduced after such treatment. Clinical improvement was so great that the patient refused operative intervention. Case III, studied 40 days after the second course of roentgen ray therapy (a total of 2700 r units was given each time to the pituitary body), received no apparent clinical benefit from these treatments, the basal metabolic rate was unchanged, and the calcium excretion remained essentially the same.

All patients except Case V showed negative phosphorus and nitrogen balances. The average phosphorus loss was somewhat greater than might be expected considering the N/P and Ca/P ratios.

At the suggestion of Dr. J. H. Means, Case III received Lugol's solution in order to determine if it would have any effect on the basal metabolic rate and the calcium and phosphorus excretions. The iodine solution was given 10 days prior to, as well as during, the period of observation. Table IV shows that it exerted no effect. This

TABLE IV

*The basal metabolic rate and the average calcium and phosphorus excretion per 3-day period in Case III before and during the administration of Lugol's solution*

	Number of 3-day metabolic periods averaged	Calcium			Phosphorus			Basal metabolic rate
		Urine	Feces	Total	Urine	Feces	Total	
		grams	grams	grams	grams	grams	grams	
Before	4	0.57	0.34	0.91	1.51	0.63	2.04	+26, +11, +15, +7
During	3	0.54	0.43	0.97	1.60	0.63	2.23	+1 to +13

is contrary to Friedgood's experience with guinea pigs receiving injections of anterior pituitary ex-

tract (18). He found that the basal metabolic rate was lowered when Lugol's solution was administered to these animals.

That the observed increased calcium excretion is not the result of an increased metabolic rate is well illustrated in Table I. Here an increased calcium excretion is seen in Case II, despite the fact that the basal metabolic rate was —22. Likewise, an increased calcium excretion was observed in one case of Cushing's basophilism with a very low metabolic rate (19, 20). Furthermore, it has been shown that the elevated metabolism produced by leukemia and fever is not accompanied by an increased calcium excretion (21).

#### DISCUSSION

From the data presented it is apparent that the calcium and phosphorus metabolism of acromegals differs from that of normal individuals. This abnormality is characterized by an increased urinary calcium and phosphorus output, the serum calcium and phosphorus and the fecal calcium and phosphorus being normal. This metabolic finding is not encountered in either hyperthyroidism or hyperparathyroidism. In the former, both the urinary and fecal calcium and phosphorus excretions are increased, and the blood values are normal. In hyperparathyroidism, the increased urinary calcium and phosphorus excretions are associated with a hypercalcemia and a hypophosphatemia. From our data it would seem unlikely that the observed increased urinary calcium excretion of the acromegalic is the result of stimulation of the thyroid and the parathyroid glands by the pituitary. However, it is impossible to rule out such a relationship. This same pituitary effect is observed in Cushing's basophilism syndrome (due to a basophilic adenoma), a disease characterized by a low metabolic rate (20, 22). From the present data it would appear wiser to assume that the anterior pituitary gland influences the calcium metabolism and that no relationship with other glands of internal secretion has been established. Thus it would appear that another endocrine gland is capable of influencing the rate of calcium exchange.

#### CONCLUSIONS

Patients with acromegaly have an abnormal calcium and phosphorus metabolism characterized

by an increased urinary excretion of these minerals, although the blood and fecal calcium and phosphorus values are normal. These increased excretion rates roughly parallel the intensity of the disease but are not dependent upon an elevated basal metabolic rate.

Roentgen ray therapy of the pituitary gland caused a reduction in the urinary calcium and phosphorus excretion in one patient and was without effect in another. The administration of Lugol's solution to one patient did not alter the basal metabolic rate or the calcium and phosphorus metabolism.

It is therefore concluded that the pituitary gland must be considered one of the factors capable of altering the calcium and phosphorus metabolism.

#### PROTOCOLS

*Case I*, Mrs. M. T. B., a 33-year-old, married housewife, was admitted to the hospital on March 24, 1927, because of amenorrhea and acromegalic features. Her past history was essentially negative. She had been married 14 years and had 2 children living and well. There had been no other pregnancies and no miscarriages. Six years before her menses had ceased. About this same time she noticed that the features of her face were coarser, her lips thicker, and that she required a larger hat. Definite enlargement of the hands and feet was also observed. All these changes gradually increased up to the time of her entrance to the hospital. She further complained of loss of libido, weakness and frontoparietal headaches which occurred about once a month, lasted 24 to 48 hours, and were never accompanied by nausea and vomiting. There was no history of blurring or dimness of vision.

On physical examination the patient was found to be a large-framed woman showing coarse facial features, thick, moist skin, prominent superciliary ridges, a large, broad nose, large ears, marked prognathism, widely separated teeth and an enlarged tongue. The eyes were slightly more prominent than normal. The margins of the discs were blurred. Vision was normal. The visual fields were normal. The isthmus of the thyroid was enlarged. Examination of the heart, lungs and abdomen was negative. The blood pressure was 180/100. A slight right dorsal and left lumbar scoliosis was present. The uterus was slightly enlarged and retroverted. The hands and feet were larger and thicker than normal; the fingers and toes were broad. A neurological examination was negative.

Laboratory examinations were as follows: Routine blood and urine examinations were negative. Renal function tests were normal. Blood Wassermann was negative. The basal metabolism test was +27 per cent. Sugar tolerance test (100 grams of glucose):

Time	Blood sugar mgm. per 100 cc.
Fasting .....	99
30 minutes .....	200
60 minutes .....	178
150 minutes .....	158

Roentgenograms showed a thickened skull, a definitely enlarged sella turcica with some destruction of the floor and the posterior clinoid processes, enlarged sinuses, prominent mandible with separated teeth, spade-like hands and feet, with some tufting of the terminal phalanges.

Metabolic studies were made before the patient was given a course of deep x-ray therapy. The x-ray treatment resulted in disappearance of the headaches, increase in strength and a better state of wellbeing without any obvious change in the acromegalic features. She was re-admitted to the hospital on February 14, 1928, for further metabolic studies. She had been symptom-free since her first entrance. Physical examination at this time showed no new findings. The sugar tolerance curve was almost identical with that of the previous year, and the basal metabolism test was +25 per cent.

Impression: Acromegaly with moderate to severe hyperpituitarism probably due to an acidophilic adenoma of the anterior lobe of the pituitary.

*Case II*, Mr. M. K., a 30-year-old, unmarried, Jewish upholsterer, was referred to the hospital on April 4, 1927, for study because of obvious acromegalic features. The patient had always been considered "a weak and sickly child." His past history was irrelevant except for typhoid fever at 21 years of age. It was difficult to determine the exact time of onset of his various symptoms. These were weakness, dizziness, headaches, easy fatigability, drowsiness, increased appetite and constipation. Enlargement of the hands and feet was first noticed 4 years previously. His weight of 174 pounds had remained unchanged during these 4 years.

Physical examination revealed a large-framed man with coarse features, coarse, moist skin, normal distribution of body hair, a widened nose, thick lips, an enlarged tongue, separated teeth and prognathism. The tonsils were enlarged. Examination of the heart, lungs and abdomen was negative. The blood pressure was 130/85. His hands were enlarged and spade-like in appearance. His feet were enlarged and showed broad toes. Neurological examination was normal.

The laboratory examinations were as follows: Routine blood and urine examinations were normal. Blood Wassermann was negative. The basal metabolism test was -22 per cent. Sugar tolerance test (100 grams of glucose):

Time	Blood sugar mgm. per 100 cc.
Fasting .....	91
30 minutes .....	125
60 minutes .....	140
120 minutes .....	127
180 minutes .....	115

Roentgenograms showed definite enlargement of the sella turcica with some erosion of the clinoid processes, enlarged sinuses, hypertrophy of the mandible, separation of the teeth, tufting of the terminal phalanges and broad metacarpals.

Impression: Acromegaly, probably due to a slow growing acidophilic adenoma of the anterior lobe of the pituitary or one which had ceased to grow. The patient had hyperpituitarism, but the basal metabolism test and sugar tolerance curve suggested that the activity of the pituitary was much less marked at the time he was studied than it had been.

*Case III*, Mr. A. R. J., a 56-year-old, married, unemployed insurance agent, first entered the hospital in May, 1936, because of acromegalic features. His past history was essentially negative except that he had suffered from mild Raynaud's disease for 20 years. He had been married 26 years and had 3 children. Ten years prior to entry, a friend first called attention to his acromegalic features. The patient thought they had become more marked since then, although he denied increase in size of head, hands and feet. Two years before, he first noted separation of the teeth, a dorsal kyphosis and forward bulging of the chest. His other complaints were weakness, loss of energy, easy fatigability, a voracious appetite, increased sweating and slight progressive blurring of vision.

On physical examination the patient was found to be a tall, well-developed, somewhat undernourished man. He had a well-defined dorsal kyphosis. His chest was barrel-shaped. He had a warm, moist, thick skin, moderately enlarged superciliary ridges, broadening of the nose, moderate prognathism, separation of the teeth and enlargement of the tongue. Examination of the eyes revealed haziness of the lens, marked myopia and upper bitemporal quadrantic anopsia. The thyroid was not enlarged. Examination of the heart and lungs was negative. The blood pressure was 120/90. The liver and spleen were felt one finger's breadth below the costal margin. The right kidney pole was palpable. The genitalia were normal. The hands and feet seemed slightly enlarged, the fingers and toes being broader than normal. Neurological examination was negative.

The laboratory examinations were as follows: Routine blood and urine examinations were normal. Fasting non-protein nitrogen and blood sugar were normal. Blood Wassermann was negative. The basal metabolism tests were +26, +11, +15 and +7 per cent. Sugar tolerance test (100 grams of glucose):

Time	Blood sugar mgm. per 100 cc.
Fasting .....	97.6
30 minutes .....	114.0
90 minutes .....	170.4
180 minutes .....	138.0
240 minutes .....	112.0

Roentgenograms showed a thickened skull, an enlarged sella turcica, enlargement of the sinuses, prominent man-

dible with separation of the teeth and tufting of the terminal phalanges of the hands and feet.

Metabolic studies were made at the time of the first admission. The patient was then discharged home for 10 days on the same low calcium diet. During this 10-day period and the subsequent metabolic study period, he received 10 minims of Lugol's solution each day. The basal metabolic rates during this period varied from +1 to +13. The third metabolic study was made 40 days after the last of two courses of deep x-ray therapy. During each course of treatment he received a total of 960 r to each of the temporal and frontal regions. The eight basal metabolism tests during the third metabolic period varied from +5 to +24. The patient's condition was seemingly unchanged as a result of the treatment given.

Impression: Acromegaly with slight to moderate hyperpituitarism probably due to an acidophilic adenoma of the anterior lobe of the pituitary.

*Case IV*, Mrs. M. D., a 34-year-old, married, Greek housewife, entered the hospital January 17, 1928, complaining of amenorrhea. Her appendix had been removed 15 years before. Otherwise her past history was essentially negative. The patient's family was of large stature and she was considered a large woman prior to the onset of her present illness. Hypertrichosis, a family characteristic, had been present for 15 years. Three months after her marriage, 8 years before admission, she had first noted swelling of the hands and fingers. She had had 6 miscarriages the first 2 years of married life. She had never become pregnant after "radium treatment of the womb" in 1922. Following the radium treatment amenorrhea gradually increased so that she menstruated only twice a year, and at such times her menstrual flow was very scanty. During the previous 3 years, the hypertrichosis of her face had increased, her facial appearance had changed, her hands and feet had definitely enlarged, and she frequently suffered from bitemporal headaches which were often accompanied by nausea, vomiting, diplopia and blurred vision. She had frequent epistaxis which she considered as evidence of vicarious menstruation, yet no relation to her regular menstrual periods could be established. She also complained of great fatigability, weakness, hyperhidrosis, intermittent pains in the extremities, loss of libido, occasional precordial pain and a tendency to constipation. A photograph taken in 1925 showed definite acromegalic features, whereas one taken 5 years before did not. She had gained 50 pounds in the previous 7 years.

Physical examination revealed a large-framed woman of good muscular development who showed obvious acromegalic features such as coarse, moist skin, hypertrichosis of the face, arms and legs, prominent superciliary ridges, a widened nose with large nares, thick lips, separated teeth, a prognathic lower jaw, large, thick hands and feet, broad fingers and toes. Examination of the heart was negative except for a systolic murmur heard over the entire precordium but loudest at the apex. The blood pressure was 118/76. Examination of the

eyes showed indistinct disc margins and bitemporal upper quadrantic anopsia.

The laboratory examinations were as follows: Routine blood and urine examinations were considered normal. Blood and spinal fluid Wassermann tests were negative. The basal metabolism test was +17 per cent. Sugar tolerance test (100 grams of glucose):

Time	Blood sugar mgm. per 100 cc.
Fasting .....	114
45 minutes .....	145
150 minutes .....	167

Roentgenograms disclosed an unusually thick skull, particularly of the frontal bones, very large sinuses, marked hypertrophy of the mandible, a definitely enlarged sella turcica, with thinning and irregularity of the posterior clinoid processes. X-ray examination of the gastrointestinal tract showed a large, dilated, atonic stomach with sluggish peristalsis; the rectum, sigmoid and ascending colon were very much dilated with absent haustral markings; the cecum was large but showed normal haustrations. The patient was studied before a course of deep x-ray therapy was given. Following this treatment, the patient's headaches were less frequent and her weakness seemed less marked.

Impression: Acromegaly with slight hyperpituitarism probably caused by an acidophilic adenoma of the anterior lobe of the pituitary gland.

*Case V*, Mrs. R. M., a 46-year-old, white, widowed, housekeeper, entered the hospital October 19, 1928, complaining of headaches. Her past history was not significant. There was no family history of gigantism. She had 2 children living and well; there had been no other pregnancies and no miscarriages. Six years before she was told that her facial appearance had changed. Two years later enlargement of the hands and feet was sufficiently marked to require larger gloves and shoes. The changes noted in her facial features, hands and feet gradually increased up to 6 months prior to entry. Amenorrhea had been present for 3½ years. In 1924 she first noticed enlargement of the thyroid. She was seen in the thyroid clinic that year. Her symptoms were hot flashes, nervousness, diarrhea, palpitation, hyperhidrosis and occasional frontal headaches. Examination at that time showed typical acromegalic features but no signs suggesting exophthalmic goiter, although her basal metabolism was +30 per cent. The sella turcica appeared normal on x-ray examination. Her vision and visual fields were normal. Three weeks before admittance to the hospital, she developed a severe occipitofrontal headache associated with nausea, vomiting, diplopia and dimness of vision. This attack lasted 2 days. She had 4 other such attacks, each being less severe and of shorter duration.

Physical examination revealed a medium sized woman, fairly well developed and nourished. The following acromegalic features were present: prominent superciliary

ridges, a broad nose, a dry, thick skin, moderately thick lips, an enlarged tongue, moderate prognathism, with moderate separation of the teeth, enlarged, spade-like hands and enlarged feet. The eyes were slightly prominent. There was paralysis of the left external rectus muscle. Examination of the fundi showed slight haziness of the upper right disc margin. The tonsils were enlarged and appeared cryptic. The thyroid was definitely enlarged; no nodules were felt and no thrill or bruit was present. The heart was normal except for a systolic murmur at the apex. The blood pressure was 120/80. The lungs were negative. The abdominal examination was negative. Neurological examination was normal.

The laboratory examinations were as follows: All urine examinations showed a trace of albumin; no other abnormalities were found. Red blood cell count 4,200,000; white blood cell count 8,100; hemoglobin 70 per cent. The blood smear showed 53 per cent polymorphonuclears, 39 per cent small lymphocytes, 7 per cent large mononuclears, and 1 per cent eosinophils. Blood Wassermann was negative. The basal metabolism test was -24 per cent. Roentgenogram of the sella turcica showed definite enlargement when compared with that taken in 1924. There was slight tufting of the terminal phalanges.

Impression: Acromegaly probably due to a slow-growing acidophilic adenoma of the anterior lobe of the pituitary. Hyperpituitarism had probably existed at some previous time but the clinical studies at the time the patient was studied suggested decreased pituitary activity.

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# ACID-BASE BALANCE, RENAL FUNCTION, AND GASTRIC SECRETION DURING HYPOCHLOREMIA IN THE DOG<sup>1</sup>

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The mechanism of the severe nitrogen retention seen in extrarenal conditions such as pyloric stenosis or intestinal obstruction has not been established conclusively. Some investigators attribute it to transient renal insufficiency of a purely functional character (1, 2). Others (3, 4) believe that renal function is essentially unaffected and relate the azotemia directly to the hypochloremia. However, marked nitrogen retention has been observed frequently in the presence of normal or high levels of plasma chloride (5, 6). Kerpel-Fronius (7), furthermore, has shown experimentally that azotemia is not a complication of hypochloremia in the absence of dehydration and also that azotemia does develop in dehydration without hypochloremia. The purpose of the present study was to clarify the problem of extrarenal azotemia by studying the effects of a gradually induced hypochloremia on the acid-base equilibrium and renal function in the dog. The investigation also includes an analysis of the histamine-stimulated gastric secretion removed during these experiments.

## METHOD OF STUDY

Chloride deprivation was gradually produced by the intermittent withdrawal of the gastric contents. It was desirable to remove the gastric juice under conditions otherwise as nearly normal as possible. For this purpose two healthy female dogs were operated upon under ether anesthesia. A special gold-plated cannula<sup>2</sup> was intro-

duced into the stomach and was brought through the abdominal wall to the exterior. After recovery from the operation, the dogs were placed on a liberal diet with added vitamins. The chloride content of the food was carefully determined and the intake of salt was maintained at a low level almost constantly except for one short period during which food with a higher salt content was inadvertently used. The chloride intake in dog 14 (weight 7.6 kgm.) averaged 315 mgm. daily, excluding seven days when the daily intake approximated 499 mgm. The chloride intake in dog 35 (weight 11.0 kgm.) averaged 467 mgm. daily, except for one week during which the daily amount rose to 746 mgm. Both dogs received a liberal allowance of chloride-free water which they drank freely throughout the experiment. In dog 14 the intake averaged 237 cc. daily for most of the experimental period; this amount was increased to 366 cc. daily during the final four weeks. Dog 35 received 331 cc. daily at first. The intake was then increased to 462 cc. and during the last seven days averaged 975 cc. (including 100 cc. of 2½ per cent sodium citrate and 300 cc. 5 per cent glucose in distilled water intravenously).

The weight of the animals was checked every seven to fourteen days. The serum chloride, carbon dioxide and pH, and the blood urea nitrogen were determined at weekly intervals (8); in addition, renal function in dog 35 was measured by the urea clearance method of Van Slyke (9). The urine was collected for two hours in these studies. After a control period of several weeks, the removal of gastric contents was begun. The dogs were trained to stand quietly on a table and the secretion of gastric juice was stimulated with hourly subcutaneous injections of histamine, the average dose ranging from 0.33 to 0.50 mgm. in dog 14 and from 0.50 to 1.0 mgm. in dog 35. The contents were collected in glass Soxhlet

completely fills the lumen of the cannula. Both the cannula and the plunger are gold-plated. The introduction into the stomach is simple. An incision is made in the anterior surface of the antrum just long enough to permit insertion of the flange. Two pursestring sutures are taken in the stomach around the shaft of the cannula and the omentum is wrapped around the shaft. The distal end of the cannula is brought to the outside through a stab wound in the abdominal wall. There has been no leakage with subsequent excoriation of the skin and no loss of gastric juice in dogs prepared by this method. On withdrawal of the cap and plunger there is an immediate flow of gastric contents undiluted by materials which usually accumulate in the shaft of a cannula with a simple cap.

<sup>1</sup>Read before the Central Society for Clinical Research, November 1, 1940.

<sup>2</sup>The gastrostomy cannula employed was devised by Dr. Alfred J. Klein of the Department of Medicine and is similar to that described by Dragstedt (Surg., Gynec., and Obst., 1933, 56, 799). In the quantitative collection of gastric juice intermittently over a period of weeks, the addition of a plunger to Dragstedt's cannula has proved useful. The cannula proper consists of a brass tube with an internal diameter of 1.1 cm. and is 7.5 cm. in length. The circular flange at the proximal end has a diameter of 3.5 cm. Attached to the cap, which screws on the distal end of the cannula, is a closed hollow brass cylinder so constructed in diameter and length that it



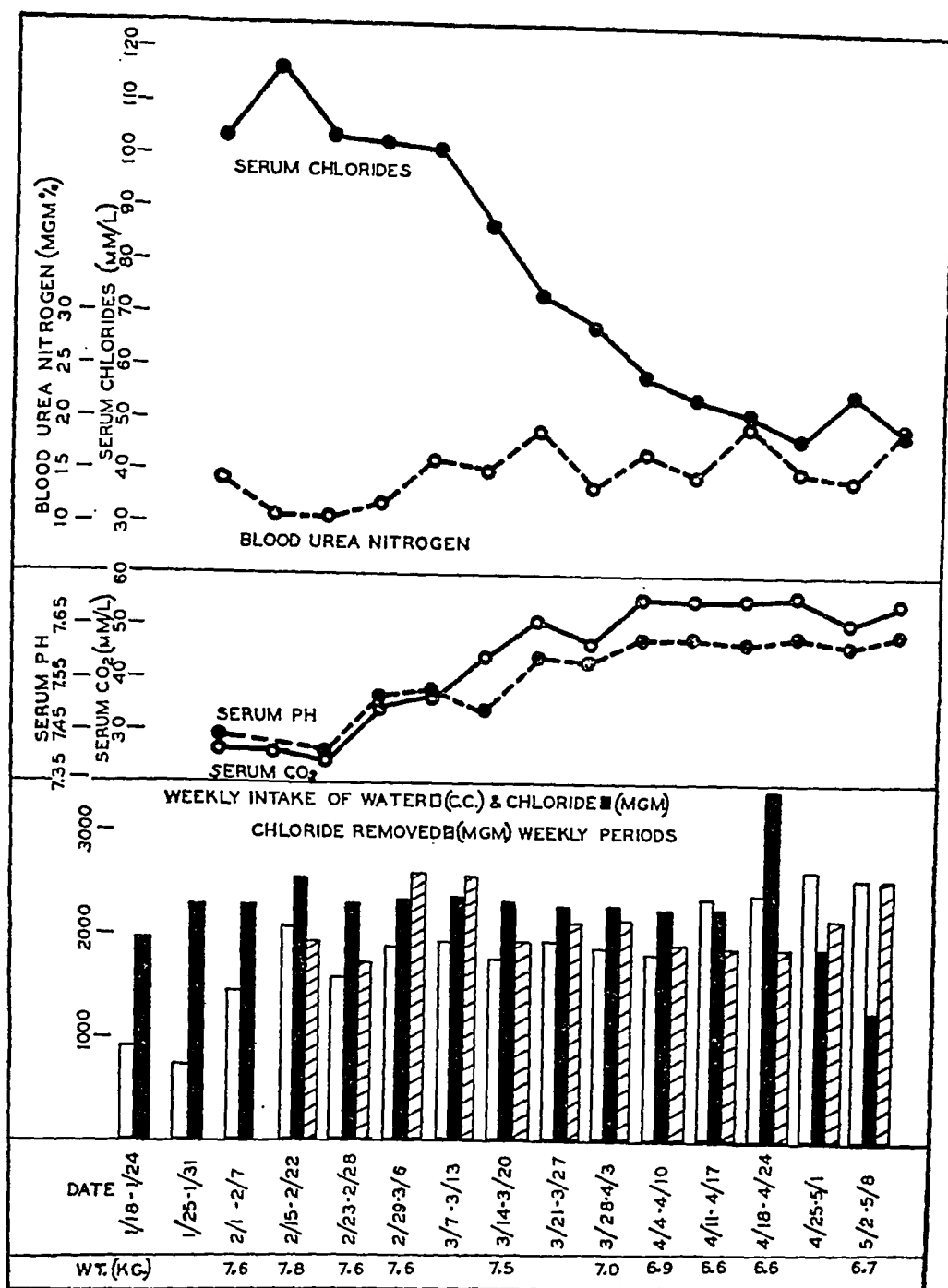


FIG. 1. THE EFFECT OF GRADUAL CHLORIDE DEPLETION ON THE ACID-BASE BALANCE AND BLOOD UREA NITROGEN IN DOG NUMBER 14

flasks, after removal of the screw-cap plunger from the cannula, for three hours each day. Food and liquids were placed in the cages after each collection. Accurate records were kept of the volume of secretion removed daily; the pH was determined with a Beckman pH meter; the chlorides were measured by the method of Van Slyke and Sendroy (8). It was impossible to prevent the occasional accumulation of saliva in the stomach; regurgitation of bile, however, was infrequent.

#### RESULTS

The gastric contents were withdrawn intermittently from dog 14 over a period of seventy-four

days. It will be noted (Figure 1) that the serum chloride decreased gradually from an average of 105.5 mM. per liter to the low value of 46.8 mM. per liter, a reduction of 56 per cent. The serum carbon dioxide rose to a peak of 56.2 mM. per liter, and the pH increased to a level of 7.63. The total anion concentration (Cl and HCO<sub>3</sub>) decreased from an average of 135 mM. per liter, when the acid-base balance was normal, to 103.1 mM. per liter as the hypochloremia became severe. Despite the severe hypochloremia and alkalosis,

TABLE I  
Gastric secretion during hypochloremia

	Gastric juice (daily values)		
	Volume	pH	Chloride
	cc.		mM. per liter
Dog 14:			
a. Period of normal blood chloride (103.6-101.2 mM. per liter)	70-150	1.20-1.43	132-148
	average 99	average 1.30	average 138
b. Severe hypochloremia (55.2-46.8 mM. per liter)	56-124	1.40-2.70	92-126
	average 84	average 1.87	average 117
Dog 35:			
a. Period of normal blood chloride (108.4-98.4 mM. per liter)	87-170	1.18-2.41	132-148
	average 121	average 1.43	average 140
b. Severe hypochloremia (56.4-38.9 mM. per liter)	46-159	1.45-1.65	112-140
	average 123	average 1.51	average 131

the rise in blood urea nitrogen was slight, ranging from 14.0 to 19.7 mgm. per cent during the period of chloride loss. It will be noted (Table I, Figure 2) that the volume and chloride content of the gastric juice decreased moderately while the pH rose slightly. Weight loss amounted to 1.1 kgm., or 14 per cent of the body weight, and oc-

curred chiefly during the latter stages of the experiment. Weakness gradually increased and on the seventy-fourth day the withdrawal of gastric juice was discontinued. The dog was found dead in its cage several days later. At necropsy the lungs were congested but there was no evidence of pneumonia. The kidneys showed early post-mortem changes. The glomeruli and tubules were normal on microscopic examination, although considerable protein precipitate was present in the glomerular spaces and in the tubules. Calcium was not found in the kidney, using von Kossa's silver nitrate stain. One parathyroid gland was successfully located and appeared entirely normal on histologic examination.

The gastric contents were withdrawn intermittently from dog 35 for eighty-six days. The serum chloride, carbon dioxide and pH remained normal for thirty-nine days, by which time 13,510 mgm. of chloride had been removed (Figure 3). In

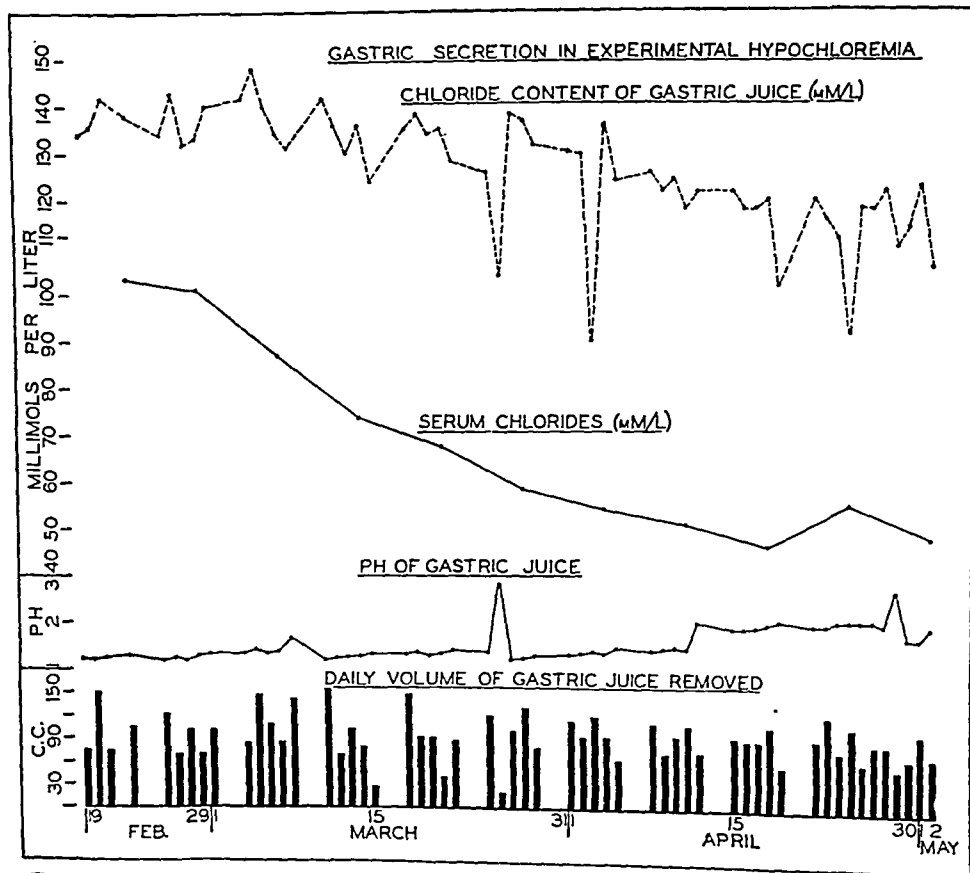


FIG. 2. EFFECT OF GRADUAL HYPOCHLOREMIA ON VOLUME, pH, AND CHLORIDE CONTENT OF HISTAMINE-STIMULATED GASTRIC SECRETION IN DOG NUMBER 14

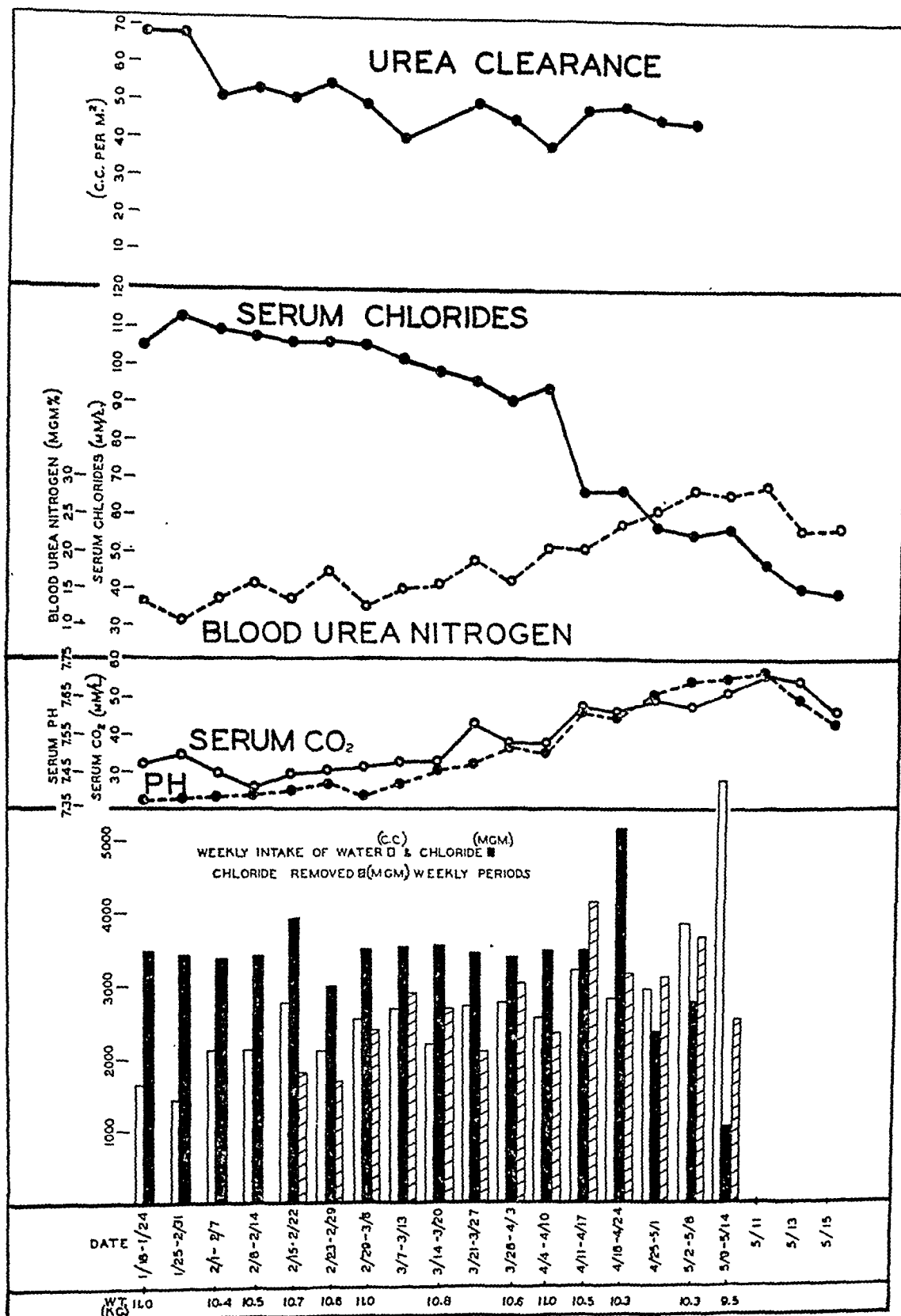


FIG. 3. THE EFFECT OF GRADUAL CHLORIDE DEPLETION ON THE ACID-BASE BALANCE, BLOOD UREA NITROGEN, AND UREA CLEARANCE IN DOG NUMBER 35

the following week the serum chloride decreased appreciably and eventually diminished from an average of 107.3 mM. per liter to the low level of 8.9 mM. per liter, a reduction of 64 per cent. The serum carbon dioxide rose to a height of 7.5 mM. per liter and the pH to a level of 7.72. During the final two days the serum carbon dioxide and pH decreased despite the progressive hypochloremia. The total anion concentration ( $\text{Cl}$  and  $\text{HCO}_3$ ) diminished from an average of 31 mM. per liter, when the acid-base balance was normal, to the very low value of 82.1 mM. per liter during the final stages of the hypochloremia. The blood urea nitrogen in this animal reached a peak of 28.6 mgm. per cent. This moderate elevation of the blood urea nitrogen is in marked contrast to the high values usually found in acute hypochloremia and alkalosis. The

values ranged from 10.9 to 17.1 mgm. per cent when the serum chloride was normal and from 16.8 to 28.6 mgm. per cent during the hypochloremia. The total plasma nitrogen, non-protein nitrogen, and plasma proteins were normal several hours before death, the values being 10.14 grams per liter, 0.40 grams per liter, and 6.09 grams per cent, respectively. Excretion of ingested water was delayed in both dogs as the chloride loss was intensified. However, after diuresis appeared, urine volumes during the hypochloremia closely approximated those obtained when the acid-base balance was normal. The urine volumes in dog 35 averaged 1.72 cc. per minute during the control period and 1.46 cc. per minute during chloride deprivation. Urine collections (two-hour periods) were delayed until diuresis occurred. The urea clearances remained normal throughout the ex-

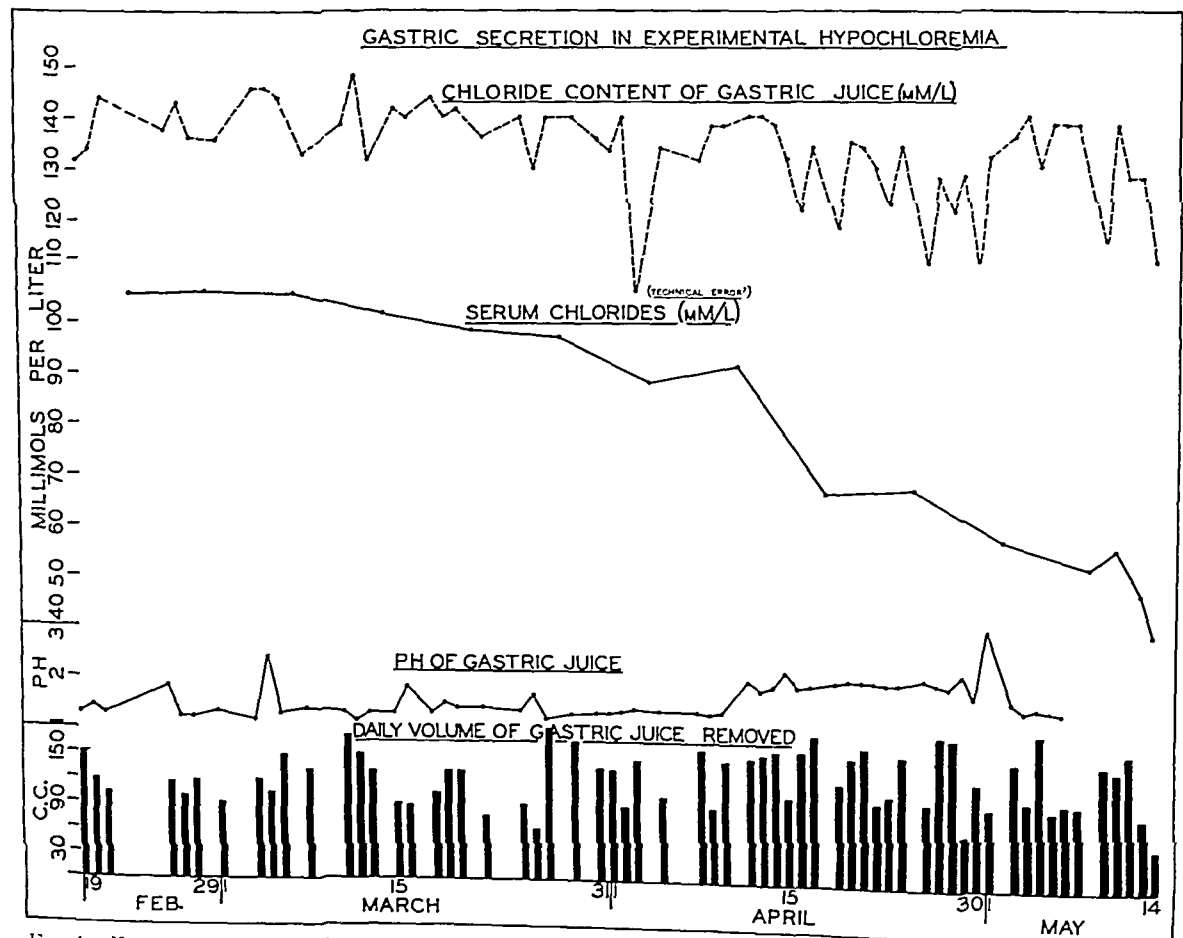


FIG. 4. EFFECT OF GRADUAL HYPOCHLOREMIA ON VOLUME, pH, AND CHLORIDE CONTENT OF HISTAMINE-STIMULATED GASTRIC SECRETION IN DOG NUMBER 35

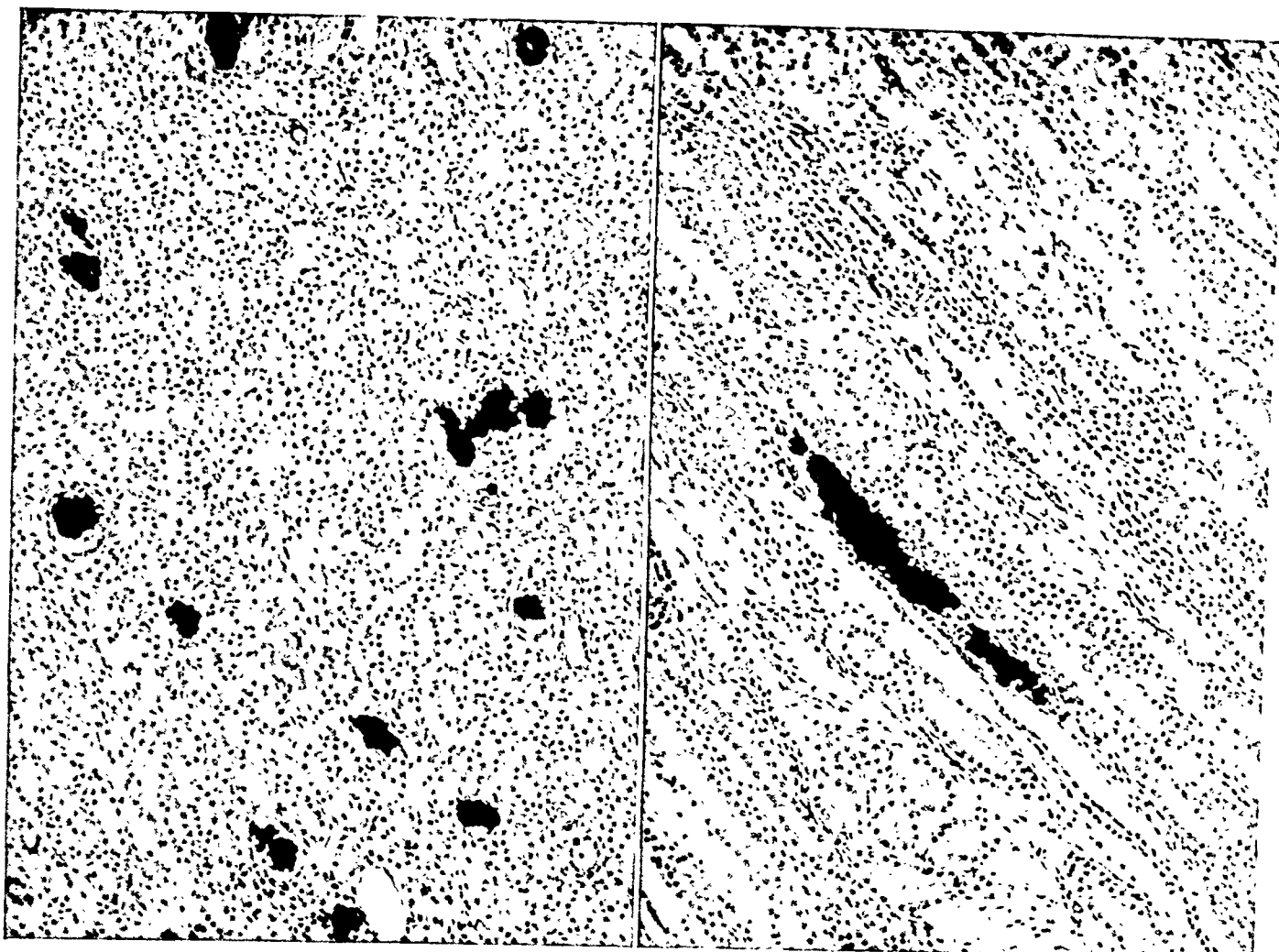


FIG. 5. PHOTOMICROGRAPH DEMONSTRATING PRESENCE OF CALCIUM PRECIPITATE IN RENAL COLLECTING TUBULES IN DOG NUMBER 35

periment, ranging from 68.9 cc. to 41.8 cc. of blood per square meter when the serum chloride was normal, and from 50.9 cc. to 47.2 cc. during the chloropenia, except for one value of 38.7 cc. These results do not differ greatly from the average urea clearances reported for the normal dog by other authors. Rhoads *et al.* (10) obtained an average clearance of 49.8 cc.; Ralli *et al.* (11), 57.7 cc.; and Jolliffe and Smith (12), 55.3 cc. In dog 35 the average daily volume of gastric juice was unchanged, the pH rose slightly, and the decrease in chloride content was comparatively small (Table I, Figure 4). Dog 35 lost 1.5 kgm., or 13.5 per cent body weight, almost entirely during the last seven days of the experiment. Muscular hyperirritability developed on the eighty-eighth day and the animal died several hours later. At necropsy the glomeruli were normal and there was slight focal tubular atrophy with cortical scarring. The lumina of many of the collecting tu-

bules were filled with blue staining masses which proved to be calcium (Figure 5). Analysis of the skin, skeletal muscle, liver, and kidney in both dogs revealed a markedly reduced chloride content. Direct measurements of the blood sodium were not made in these experiments. However, the fact that the sum of the anions ( $\text{Cl}$  and  $\text{HCO}_3$ ) was much below normal indicates a decreased concentration of cation, *i.e.*, sodium.

#### DISCUSSION

Inasmuch as the gastric juice is derived from water and electrolytes taken from the blood plasma, the continued loss of gastric secretion involves a considerable loss of chloride and a much smaller but significant depletion of sodium. The loss of anion ( $\text{Cl}$ ) is replaced by an extension of bicarbonate, and therefore does not alter the total electrolyte value at first, whereas loss of base is not replaceable except from the diet and, more-

over, carries with it an equivalent quantity of bicarbonate ion. The deficit of base is accompanied by a corresponding loss of extracellular water, leading to an extensive reduction in the volume of extracellular fluid (13). Severe nitrogen retention usually accompanies such acute disturbances in the plasma electrolytes and fluid (14, 15, 16). Experimentally, Dragstedt and Ellis (17) found an elevation of the urea nitrogen from 12 to 154 mgm. and of the non-protein nitrogen from 27 to 180 mgm. when the whole blood chloride decreased from 300 to 108 mgm. of chloride. The pathogenesis of the azotemia has not been clearly understood, although several explanations have been offered. Blum attributed the rise in blood urea to reabsorption of urea through the tubules of the kidney in order to raise the subnormal osmotic pressure of the plasma. Urea, however, is a crystalloid and, even if reabsorbed, would not regulate the osmotic pressure of the plasma as originally conceived for it diffuses freely through all the tissues of the body. As McCance (15) points out, nothing can act as an efficient substitute for an extracellular electrolyte unless its distribution is limited to the extracellular fluids.

A second cause which has been given for the development of extrarenal azotemia is the increased protein catabolism of dehydration. Numerous investigators (18, 19) have shown that deprivation of water alone may accelerate the breakdown of protein. McCance (20) found during acute salt deficiency produced by diet and sweating that, with a reduction in the volume of body fluids of 28 to 38 per cent, the subjects were in negative nitrogen balances. Both in clinical and experimental salt deficiency the loss of chloride is abrupt and is rapidly followed by a marked loss of body water. The nitrogen retention, therefore, may be explained simply on the basis of excessive protein breakdown associated with anhydremia (21, 22, 23). The azotemia in hypochloremia has been attributed also to impaired renal function secondary to dehydration (24, 25). This concept is supported by the findings of Landis *et al* (26) in that the mild elevation of blood urea nitrogen following restriction of sodium chloride was accompanied by a slightly diminished average twenty-four-hour urea clearance. The conventional one-hour clearances were normal. McCance and Widowson (27) found a decrease of the creatinine,

sucrose, inulin, and urea clearances during acute salt deficiency. These decreases were attributed to diminished glomerular filtration and to increased tubular reabsorption of urea. Eventual reduction of the volume of blood plasma by the process of dehydration causes physical changes in the blood and in the mechanics of its circulation which greatly reduce volume flow through the kidneys, with the result that accuracy of renal function is impaired. These changes include: increased colloidal osmotic pressure; increased blood viscosity, decreased blood volume; a reduction in the number of "active glomeruli"; and a decrease in the rate of circulation. Regardless of the specific mechanisms operative, it is apparent that the basic renal disturbance is not one of primary or secondary renal *disease* but of impaired renal *function* secondary to dehydration.

This study, in contrast to the previous investigations, indicates that a gradually induced hypochloremia is not necessarily accompanied by severe nitrogen retention. Ambard and his associates (28) and Hiatt (29) have made similar observations. The decisive factor which determines the extent of azotemia during hypochloremia appears to be the degree and rapidity of dehydration associated with the chloride loss. The extracellular fluid was not measured but, presumably, was diminished. Evidence for its decrease is to be found in the significant loss of weight sustained by both dogs and also in the established fact that loss of body fluid occurs with a decrease in serum electrolytes (30). Since the animals drank considerable amounts of chloride-free water daily, it would appear that dehydration did not exist in the sense of deprivation of water, but was present in the sense that loss of body fluid accompanied the loss of electrolytes. However, the dogs apparently were able to make rather satisfactory adjustments to severe changes in the electrolyte balance during most of the experiment. The normal urea clearances and the finding of normal nitrogenous constituents in the blood several hours before death are evidence of adequate renal function. The mild elevation of blood urea nitrogen may be considered as indicative of a decrease in renal function too small to be detected by the usual clearances, as suggested by the work of Landis. The explanation of such an assumed impairment of renal function, however, is not easily hypothe-

sized. The urinary volumes were excellent, although we observed, as have other workers, an unexplained delay in water diuresis in these animals.

The calcium precipitate observed in the renal collecting tubules in dog 35 is of considerable interest since it is not usually present in the normal dog and was not found in dog 14. This condition was described originally by Nazari (31) in 1904 in two patients with prolonged vomiting secondary to pyloric stenosis. Many similar reports subsequently appeared (32, 33, 34) but the mechanism of the condition is not entirely clear. Calcium salts are not infrequently deposited in necrotic kidney cells. Martz and others believe that the secretion of an acid urine during severe alkalosis makes the cell fluids of the kidney even more alkaline and thereby favors the precipitation of calcium. Hatano (35) was able to prevent this complication during hypochloremia by the intravenous injection of sodium chloride which tended to correct the acid-base balance. The use of sodium bicarbonate proved ineffective. Gömöri and his co-workers (36) have frequently observed fat degeneration with necrosis and calcification in the kidneys of patients dying of "hypochloremic uremia." Since the kidneys in the present experiment were normal, the calcium precipitate is probably the result of an abnormal physical chemical state in the urine.

The results of the present investigation are in complete accord with other studies in demonstrating that during periods of marked hypochloremia, the gastric mucosa is able to secrete normal amounts of highly acid gastric juice. Dragstedt and Ellis (17), working on dogs with total gastric pouches, found that the gastric glands continued to secrete in spite of a plasma chloride level lower than one-third of normal; the volume of secretion, however, was reduced and its acidity lessened. Lim and Ni (37) observed a continued secretion in dogs despite dehydration and a loss of one-half of the total body chloride. Katsch and Mellinghoff (38) and Mellinghoff and Heuschert (39) withdrew gastric juice from patients for ten hours each day for three to five days, removing up to 33 grams of sodium chloride, and produced an average fall in the whole blood chloride of 51 mgm. per 100 cc. The gastric secretion showed a 20 per cent reduction in volume without significant

change in the chloride content. The response to histamine was as good or almost as good on the last day of treatment as it had been on the first. McCance (20) found that salt deficiency produced by diet and sweating tended to reduce the concentration of acid and chloride in gastric juice but that individuals varied widely in their response. Solely, Lagen and Lockhart (40) examined three healthy men over a period of one week on a salt-deficient diet, an increased output of salt being obtained by frequent periods of sweating. They found no significant change in the free acidity or total hydrochloric acid of the gastric juice under these conditions. The degree of salt deficiency, however, was very slight. Nicol and Lyall (41) studied a group of patients with peptic ulcer in whom hypochloremia had been induced by vomiting and concluded that the gastric mucosa could still secrete normal amounts of hydrochloric acid. The blood chloride in one patient had decreased to 60 mM. per liter. Hiatt (29) has recently found that the volume and total acidity of the histamine-stimulated gastric secretion were decreased in dogs with chloride levels below 65 per cent of normal. The function of the gastric glands is apparently unique, since chloride secretion by other routes, including the skin and choroid plexus, and its excretion via the kidney are diminished or cease completely in the presence of a severe hypochloremia.

#### SUMMARY

A gradual depletion of body chloride was produced in two dogs by the intermittent withdrawal of histamine-stimulated gastric juice over periods of seventy-four and eighty-six days. The dogs received a generous diet with added vitamins and a liberal allowance of chloride-free water. The salt intake was maintained at a low level, averaging 315 and 467 mgm. daily. At weekly intervals determinations were made of the serum chloride, carbon dioxide, pH and blood urea nitrogen, and renal function in one animal was measured by the urea clearance method of Van Slyke. In dog 14 the serum chloride decreased from an average of 105.5 mM. per liter to 46.8 mM. per liter, while in dog 35 the serum chloride decreased from an average of 107.3 mM. per liter to 38.9 mM. per liter. In dog 14 the carbon dioxide rose to 56.2 mM. per liter and the pH to 7.63; in dog 35 the carbon dioxide rose to a height of 57.5 mM. per

liter and the pH to 7.72. The blood urea nitrogen in dog 14 ranged from 10.6 to 16.6 mgm. per cent when the acid-base balance was normal, and from 13.3 to 19.7 mgm. per cent during the hypochloremia. The blood urea nitrogen in dog 35 ranged from 10.9 to 17.1 mgm. per cent with a normal acid-base balance and from 16.8 to 28.6 mgm. per cent during the chloropenia. Both dogs lost approximately 14 per cent of body weight. There was indirect evidence to suggest that the blood sodium was diminished. Since both animals were allowed to drink as much chloride-free water as desired, dehydration in the ordinary sense of water deprivation was not present. The nitrogenous constituents of the blood in dog 35 were normal shortly before death. The urea clearance remained normal throughout, ranging from 68.9 to 41.8 cc. during the control period and from 50.9 to 47.2 cc. during the depletion of body chloride. The excretion of ingested water was delayed in both dogs as the loss of chloride was intensified. In dog 14 there was a moderate decrease in the volume and chloride content of the gastric juice, the pH rising slightly; while in dog 35 the volume, pH and chloride content of the gastric juice did not change significantly. Calcium precipitation was observed in the renal collecting tubules of dog 35.

#### CONCLUSIONS

1. A severe alkalosis may be induced by the gradual withdrawal of gastric contents without severe or, indeed, even marked nitrogen retention.
2. Such an alkalosis apparently does not produce renal injury detectable either by the urea clearance test or by histologic examination.
3. The secretion of hydrochloric acid by the gastric mucosa is relatively uninfluenced by a profound hypochloremia.

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# CLINICAL STUDIES ON PYRIDOXINE (VITAMIN B<sub>6</sub>)

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The isolation (1), synthesis (2) and physiological significance (3 to 9) of pyridoxine<sup>1</sup> in animal nutrition have recently been established. Clinical studies have not been very extensive. Spies and his collaborators (10) have noted that certain residual symptoms (*i.e.*, insomnia, irritability, weakness, difficulty in walking and abdominal pain) in treated pellagrins responded to pyridoxine therapy. In a case of arsenical neuritis, Vilter (11) commented on the rapid remission when pyridoxine was added to the already instituted therapy. He also observed an exacerbation when pyridoxine was discontinued. Smith and Martin (12) successfully treated three out of four patients suffering with cheilosis. In the fourth patient the addition of liver extract brought relief. These investigators state that deficiency of either riboflavin or pyridoxine may be the etiological factor primarily responsible for cheilosis or that both together are necessary in maintaining the integrity of the mucocutaneous junction. Antopol (13) noted improvement in a group of patients with pseudohypertrophic muscular dystrophy when pyridoxine was administered. Lessening in rigidity and improvement in muscle strength were noted in a group of patients suffering with Parkinsonism after the use of pyridoxine (14, 15). This improvement was observed particularly in early cases of senile Parkinson's disease.

There have been isolated reports indicating favorable response to pyridoxine therapy in both macrocytic (16) and microcytic (12) anemias. Kark and his associates (17), however, after studying the responses of six anemic patients following pyridoxine administration, did not observe any improvement in the hematological picture.

Recently, Scudi and his co-workers (18) reported a colorimetric test for the determination of

pyridoxine. They (19) found that normal rats excreted a higher percentage of the ingested vitamin when compared to rats maintained on a diet deficient in pyridoxine. Applying this test to dogs and normal humans, Scudi and his collaborators (20) found that pyridoxine is rapidly absorbed from the gastro-intestinal tract and excreted by the kidneys. In dogs, 18 per cent of the vitamin was excreted in one hour when it was administered intravenously, while 20 per cent was excreted in six hours when given orally. In thirteen healthy adults receiving 50 mgm. of pyridoxine intravenously, an average of 8.7 per cent was excreted in the urine in one hour, while 7.6 per cent was recovered in the urine in four hours after 100 mgm. were given orally.

The investigations of Scudi have provided clinicians with a test which may indicate the degree of saturation or ability of the body to utilize pyridoxine. Spies (21) recently reported clinical data which confirm the usefulness of Scudi's test. This paper is an attempt at further evaluation of the method for clinical use. An effort has also been made to determine the influence of various pathological factors on the pyridoxine status in humans.

## MATERIALS AND METHODS

Patients studied in this series were from the wards and dispensary of the New York Post-Graduate Hospital and the First Medical Division of the Welfare Hospital for Chronic Diseases. The patients were on standard balanced hospital diets. No special therapy was employed except where noted. The tests were carried out by obtaining a control specimen of urine following which 50 mgm. of pyridoxine were administered intravenously and approximately 400 ml. of water were then given orally. One hour after the administration of the vitamin another urine specimen was obtained. Patients with Parkinson's disease required catheterization in order to obtain accurately timed urine specimens. The pyridoxine determinations and calculations were carried out according to the method outlined by Scudi (19, 20).

<sup>1</sup> The term pyridoxine is used interchangeably to signify pyridoxine or pyridoxine hydrochloride.

## RESULTS

Ninety-eight tests were performed on eighty-four patients. For purposes of analysis, the patients have been grouped first, according to age and pyridoxine output; and secondly, according to specific ailment, namely: Parkinson's disease and renal insufficiency.

TABLE I

*One hour urinary output of pyridoxine in patients with normal renal function from 5 to 15 years of age after the administration of 50 mgm. of pyridoxine intravenously*

Patient	Age	Sex	Pyridoxine output	Diagnosis
			<i>per cent</i>	
Var...	9	M	24.6	Pseudohypertrophic muscular dystrophy.
Den...	6	M	20.1	Reticular cell sarcoma.
Rol...	10	M	18.5	Migraine.
Gri...	12	M	18.2	Pleuritis.
Spa...	8	F	22.2	Rheumatic heart disease.
Dan...	8	F	27.3	Chorea (Sydenham).
Fum...	5	M	22.3	Chronic tonsillitis.
Fra...	15	M	15.3	Primary muscular atrophy (juvenile type).
D'ag...	6	F	19.4	Common cold.
Pie...	6	M	25.4	Common cold.

Ten patients between 5 and 15 years of age had an average urinary output of pyridoxine of 21.3 per cent (range 18.2 to 27.3 per cent). A case of pseudohypertrophic muscular dystrophy and one of primary muscular atrophy (juvenile type) were included in this group (Table I). In

TABLE II

*One hour urinary output of pyridoxine in patients with normal renal function from 16 to 50 years of age after the administration of 50 mgm. of pyridoxine intravenously*

Patient	Age	Sex	Pyridoxine output	Diagnosis
			<i>per cent</i>	
Til...	38	M	9.4	Exfoliative dermatitis.
Bar...	43	M	9.2	Psoriasis.
Sil...	33	M	8.8	Diabetes mellitus.
Met...	47	F	6.4	Menopause.
Por...	16	F	5.2	Rheumatic heart disease.
Bor...	39	F	10.8	Essential hypertension.
McM...	46	F	8.0	Essential hypertension.
Tem...	32	M	5.8	Diabetes mellitus.
Bar...	47	M	6.4	Hodgkin's disease.
Ros...	43	M	10.4	Rheumatic heart disease.
Wol...	30	F	8.6	Spastic colon.
Smi...	33	M	11.4	Peptic ulcer.
Bre...	32	M	6.5	Spinal injury.
Fis...	34	M	3.8	Exfoliative dermatitis.
Com...	24	F	1.6	Essential hypertension.

thirteen of fifteen patients between the ages of 16 and 50 years, the excretion of pyridoxine ranged from 5.2 to 11.4 per cent, with an average output of 8.4 per cent. This approximates closely the normal figure obtained by previous investigators (20, 21) (Table II). Two patients excreted 1.6 and 3.8 per cent, respectively. In forty-five patients over 50 years of age, thirty-two were within the normal range (4.1 to 11 per cent), with an average output of 7.2 per cent. Thirteen patients were below normal, with an average output of 2.3 per cent (range 1.0 to 3.1 per cent) (Table III). Table IV summarizes the influence of age on pyridoxine output; standard deviations for each group are also indicated.

TABLE III

*One hour urinary output of pyridoxine in patients with normal renal function over 50 years of age after the administration of 50 mgm. of pyridoxine intravenously*

Patient	Age	Sex	Pyridoxine output	Diagnosis	Remarks
			<i>per cent</i>		
O'R....	74	M	7.4	Generalized arteriosclerosis.	
Fou....	58	M	4.1	Exfoliative dermatitis, renal calculus.	
Bau....	55	M	6.6	Exfoliative dermatitis.	Liver extract.
Bec....	65	M	5.2	Arteriosclerotic heart disease.	
Wal....	76	M	6.4	Chronic dermatitis.	
Dia....	63	M	11.0	Generalized arteriosclerosis.	
Coh....	53	F	8.2	Diabetes mellitus.	
Mos....	65	F	10.9	Rheumatoid arthritis.	
Pow....	68	F	4.1	Generalized arteriosclerosis.	
Sch....	70	M	6.2	Diabetes mellitus.	
Bar....	75	M	5.0	Generalized arteriosclerosis.	
Sml....	68	M	7.6	Generalized arteriosclerosis.	
Sal....	71	M	8.6	Generalized arteriosclerosis.	
Col....	63	M	7.8	Generalized arteriosclerosis.	
McC....	70	M	6.8	Alcoholism.	Brewer's yeast, Vitamin B.
Spl....	62	M	8.8	Generalized arteriosclerosis.	
Fre....	74	M	7.0	Fibroid tuberculosis.	
Spo....	79	M	8.0	Arteriosclerotic heart disease.	
She....	70	M	4.4	Generalized arteriosclerosis.	
Bey....	64	M	6.8	Chronic dermatitis.	
Con....	64	M	9.1	Generalized arteriosclerosis.	
Din....	80	M	10.0	Pernicious anemia. Generalized arteriosclerosis.	
Rog....	74	F	6.0	Arteriosclerotic heart disease.	
Dia....	64	F	6.4	Generalized arteriosclerosis.	
D'am...	53	M	8.6	Toxic adenoma of the thyroid.	
Sci....	51	M	8.4	Peptic ulcer.	
Coh....	59	M	4.8	Diabetes mellitus.	
Min....	63	M	4.6	Diabetes mellitus.	
Wal....	63	M	9.4	Exfoliative dermatitis.	
Hei....	64	M	5.2	Hypertensive cardiovascular disease.	Repeat test 24 hours, 5.4 per cent.
Tom....	62	M	9.3	Pulmonary fibrosis.	
Mat....	74	M	1.2	Arteriosclerotic heart disease with chronic cardiac decompensation.	Repeat test 24 hours, 9.4 per cent.
Cus....	65	M	2.9	Chronic bronchitis.	
Doh....	66	M	2.8	Tuberculosis of cecum.	
Rot....	63	M	2.8	Hypochondriasis.	
Boc....	67	M	1.2	Diabetes mellitus.	
Mon....	67	M	1.2	Osteoarthritis.	
Lut....	69	F	2.6	Generalized arteriosclerosis.	
Whi....	66	F	1.3	Generalized arteriosclerosis and syphilis.	
Toa....	56	F	1.0	Bronchial asthma.	
McG....	80	F	3.6	Generalized arteriosclerosis.	
Fer....	61	M	2.6	Tabes dorsalis.	
Kal....	61	M	3.5	Generalized arteriosclerosis.	Repeat test, one week later, 6.4 per cent.
Matt....	62	F	3.4	Diabetes mellitus.	

TABLE IV  
Influence of age on pyridoxine output

Group	Number of patients	Urinary output average	Deviation	
			Standard	Maximum
		<i>per cent</i>	<i>per cent</i>	
5-15	10	21.3	$\pm 3.68$	6.0
16-50	13	8.4	$\pm 2.04$	3.1
	2	2.7		
Over 50	32	7.2	$\pm 1.87$	3.8
	13	2.3		

In seven patients with Parkinson's syndrome, five had a definitely low pyridoxine urinary output, and a sixth was low normal. Six of these showed an average output of 2.5 per cent (range 0.5 to 4.6 per cent), while one had a normal output of 10.7 per cent. Tests repeated one month later showed an increase to normal in two previously low patients, while the borderline normal now showed a diminished excretion. The complaints of these patients were considered to be sequelae of previous encephalitis (Table V).

TABLE V  
Influence of post-encephalitic Parkinson's syndrome on pyridoxine output

Patient	Age	Sex	Pyridoxine output	Renal impairment	Remarks
			<i>per cent</i>		
Pin.....	55	F	10.7*	0	
Fei.....	60	F	0.6 6.6	0	Repeat test, one month later.
McG.....	51	M	2.5	0	
Gol.....	51	M	0.5	0	
Psy.....	42	F	4.6 1.6	0	Repeat test, one month later.
Bor.....	34	F	3.8 5.3	0	Repeat test, one month later.
Eng.....	30	F	3.2	0	

Average output 6 cases—2.5 per cent.

\* Not included in average.

Fourteen patients with varying degrees of renal insufficiency showed an average output of only 2.2 per cent (range 0.0 to 5.4 per cent). Twelve of these (88 per cent) gave definitely low response, while the remaining two had a low normal output (Table VI).

#### DISCUSSION

It is interesting to note that when cases of renal insufficiency are excluded, thirteen of fifteen adult

TABLE VI  
Influence of renal impairment on pyridoxine output

Patient	Age	Sex	Pyridoxine output	Diagnosis	Renal impairment	Remarks
			<i>per cent</i>			
Din	82	M	3.1 2.0	Osteoarthritis, generalized arteriosclerosis.	+	Repeat tests, two mon. hs.
Bio	74	M	1.2 1.6	Diabetes mellitus, generalized arteriosclerosis.	$\pm$	Repeat tests, one week.
Sim	71	M	3.0 2.2	Diabetes mellitus, generalized arteriosclerosis.	$\pm$	Repeat tests, one week.
Bro	70	M	1.9	Generalized arteriosclerosis.	$\pm$	
Bro	70	M	1.2	Spinal degeneration with generalized arteriosclerosis.	$\pm$	
O'Da	67	M	0.6 0.4	Primary contracted kidney.	+++	Repeat tests, two months.
Wei	64	M	1.6	Generalized arteriosclerosis.	++	
Eze	59	F	1.6	Hypertensive cardiovascular disease.	++	
Cre	55	M	3.2	Hypertensive cardiovascular disease.	+++	
Bra	51	F	0.0	Chronic diffuse glomerular nephritis.	++++	Uremia.
Ric	47	M	1.4	Chronic diffuse glomerular nephritis.	+++	
Fle	42	F	4.8 3.2	Chronic diffuse glomerular nephritis.	++	Repeat tests, three months.
Res	25	M	2.0 0.7	Chronic diffuse glomerular nephritis.	++++	Repeat tests, three months.
Kra	20	M	5.4	Chronic diffuse glomerular nephritis.	+	

Average output 2.2 per cent.

patients under 50 years of age have an average pyridoxine output of 8.4 per cent;  $\pm 2.04$  per cent, which corresponds very closely to the normal values previously reported. In individuals over 50 years of age, however, only thirty-two of forty-five patients showed an excretion of pyridoxine in the urine which approximated the normal range. The remaining thirteen patients had an average output of 2.3 per cent which is well below the accepted normal.

The increased urinary output in the patients from 5 to 15 years of age probably implies that the 50 mgm. dose is excessive in relation to body weight. Investigations to determine the optimum dose per unit of body weight will be undertaken.

While the effect of age on the urinary excretion of pyridoxine is of great interest, it apparently is not a factor influencing the low average output found in six of seven patients with Parkinson's disease. It is worthy of note that the patients with parkinsonism in this series were post-encephalitic in type. Jolliffe (14), however, found pyridoxine to be a more effective therapeutic agent in the senile type of parkinsonism than in the post-encephalitic variety.

The low output encountered in patients with renal insufficiency is not surprising, and serves to emphasize the importance of knowing the renal status of all patients.

## SUMMARY

Following the intravenous administration of a test dose of pyridoxine, the urinary excretion of this vitamin was studied 98 times in eighty-four patients. Twelve of fourteen individuals (88 per cent) under 50 years of age excreted in one hour an average of 8.4 per cent of the amount injected. Thirty-two of forty-five patients (71 per cent) over 50 years of age showed an average output of 7.2 per cent; the remaining thirteen subjects (29 per cent) excreted an average of only 2.3 per cent.

Ten patients between 5 and 15 years of age eliminated an average of 21.3 per cent of the amount of pyridoxine injected. This observation suggests the advisability of using a test dose based on body weight.

Six of seven patients with post-encephalitic parkinsonism showed a diminished output of pyridoxine averaging 2.5 per cent. Twelve of fourteen patients with varying degrees of renal insufficiency demonstrated a definite impairment in the excretion of pyridoxine, while the remaining two had a low normal output.

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# DEMONSTRATION THAT THE CELL PLASMA RATIO OF BLOOD CONTAINED IN MINUTE VESSELS IS LOWER THAN THAT OF VENOUS BLOOD

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A study of the distribution of the red cells in the circulating blood is of both theoretical and practical importance. In spite of the fact that blood taken from artery, vein or finger has the same cell plasma ratio (1, 2), certain investigators (2, 3, 5) believe that the red cells are not uniformly distributed throughout the vascular bed but that the blood from the large vessels has a higher cell plasma ratio than the blood contained in the minute vessels. Direct observation of the minute blood vessels (6) has shown that the red cells flow in the rapidly moving central portion of the stream and that there is a slow moving clear layer of plasma adjacent to the wall of the vessel. Because of the size of this peripheral layer of clear plasma in the minute blood vessels, it has been suggested that the cell plasma ratio of blood contained in the minute vessels is lower than that of blood from the large vessels.

The cell plasma ratio of the blood contained in the minute vessels has not been determined because of the difficulty in obtaining a sample of this blood. Blood collected from a vein or from the cut ends of minute vessels is representative of the blood flowing from the minute vessels but it is not necessarily representative of the blood contained within the minute vessels. The blood contained within the minute vessels consists not only of the central core of red cells and plasma which is flowing rapidly into the veins, but also of the slower moving peripheral layer of plasma.

In this study a technique for obtaining a portion of the blood actually contained within the minute vessels is described. To obtain this blood it is necessary (1) to obstruct the arterial inflow to the part, (2) to empty the blood out of the large vessels, and (3) to force the blood from the minute vessels into the veins where it can be collected. The hemoglobin concentration and protein concentration of the venous blood and of the

blood contained within the minute vessels were compared.

## METHOD

Fifteen experiments were performed on six normal male subjects. All determinations were made on fasting subjects who had rested in the horizontal position for 30 minutes. A blood pressure cuff was placed loosely on the arm, care being taken not to cause venous distention. A needle, with the point toward the wrist, was introduced into the antecubital vein distal to the cuff. In most of the experiments the vein was entered without a tourniquet. When a tourniquet was used, blood was allowed to drip from the needle for 3 minutes before proceeding. A sample of venous blood was then taken. Immediately thereafter the arterial inflow to the arm was stopped by suddenly inflating the cuff on the arm to a pressure of 300 mm. of Hg. The forearm and hand were then elevated and the blood (usually 40 to 60 cc.) was removed by a syringe. When no more blood could be obtained, an Esmarch's bandage was applied to the forearm, beginning at the wrist, and the remaining blood was milked toward the needle. About 3 to 6 cc. of blood were obtained during the application of the bandage. The protein concentration and hemoglobin concentration of the venous blood and of the blood milked from the forearm were determined. The hematocrit reading of the venous blood and that of the blood squeezed from the forearm were compared in four experiments.

The hemoglobin concentration of the blood was measured by the method of Evelyn (7). With this technique changes in hemoglobin concentration of more than 0.2 gram are significant. The protein was calculated from the specific gravity of the serum by the method of Kagan (8). In three experiments the total nitrogen was determined by the micro-Kjeldahl method, using Nessler's reagent and the Klett photoelectric colorimeter.

## RESULTS

Similar results were obtained in every experiment (Table I). The hemoglobin concentration of blood milked from the minute vessels was from 0.8 to 1.8 grams lower than the hemoglobin concentration of venous blood. In four of the experiments the protein concentration remained unchanged, and in the remainder it decreased slightly.

TABLE I

*The hemoglobin concentration and protein concentration of venous blood and of blood contained within minute vessels*

Experiment	Venous blood		Blood in small vessels		Difference	
	Hemoglobin	Protein	Hemoglobin	Protein	Hemoglobin	Protein
	grams per 100 cc.	grams per 100 cc.	grams per 100 cc.	grams per 100 cc.	grams per 100 cc.	grams per 100 cc.
1	14.5	6.7	13.5	6.7	1.0	0
2	14.5	7.0	13.6	6.9	0.9	0.1
3	14.7	7.0	13.4	6.8	1.3	0.2
4	14.4	7.2	13.2	6.8	1.2	0.4
5	15.4	6.9	14.2	6.7	1.2	0.2
6	15.0	7.1	13.9	6.9	1.1	0.2
7	14.3	6.8	13.5	6.7	0.8	0.1
8	14.4	7.0	12.6	6.6	1.8	0.4
9	13.3	6.8	12.3	6.6	1.0	0.2
10	14.7	6.8	13.6	6.6	1.1	0.2
11	13.4	6.8	12.5	6.8	0.9	0
12	14.0	7.0	13.2	6.9	0.8	0.1
13	14.3	6.9*	13.1	6.8*	1.2	0
		6.9		6.9		0
14	13.8	6.6*	13.0	6.5*	0.8	
		6.7		6.7		0.1
15	14.0	6.7*	12.9	6.4*	1.1	
		6.8		6.7		
			Average		1.1	0.15

\* Done by Kjeldahl method.

This slight fall in protein concentration indicates that fluid was entering the plasma from either the extracellular tissues or from the red cells. As the ratio of the hematocrit reading to the hemoglobin concentration remained the same in the four experiments in which it was determined, the fluid probably came from the extracellular tissues. In no experiment did sufficient fluid enter the plasma to account for more than a small portion of the decrease in hemoglobin concentration. The average decrease in protein would have had to have been approximately six times greater than actually occurred to account for the fall in hemoglobin concentration on the basis of simple dilution with protein-free fluid.

After the arterial inflow to the forearm was occluded, the blood from the forearm was removed with syringes of 10 cc. capacity. In three experiments the hemoglobin concentration and protein concentration of the blood contained in each syringe were determined. In each experiment the hemoglobin concentration of the first 20 to 30 cc. of blood removed after the application of the tourniquet was the same or slightly less than that of the control sample of venous blood. There-

after the hemoglobin concentration showed a progressive fall in successive samples of blood. The protein concentration either remained unchanged or showed an insignificant decrease.

## DISCUSSION

The fact that blood from an artery, vein or finger has, in general, the same cell plasma ratio, has caused many investigators to assume that the hematocrit reading of blood removed from the body is representative of the cell plasma ratio of the entire circulating blood. If this were true, the red cell volume could be calculated from the plasma volume and hematocrit reading. This is not true, for when the red cell volume is calculated from the plasma volume and hematocrit reading, the value obtained for the red cell volume is falsely high (2, 5). It has been shown that the error in the calculation of the red cell volume occurs because the hematocrit reading of the blood removed from the body is not representative of the cell plasma ratio of the total quantity of blood in the vascular bed. Smith, Arnold and Whipple (2) compared the value for the red cell volume obtained from the plasma volume and hematocrit reading with that obtained by the carbon monoxide and Welcker methods, and concluded that the value for the red cell volume calculated from the plasma volume and hematocrit reading was approximately 20 per cent higher than the actual red cell volume. Hahn et al. (4) determined the red cell volume of dogs by a method using radio iron. They concluded that the red cell mass in circulation determined by this method averaged about 75 per cent of the value as computed by the jugular hematocrit reading from the plasma volume (dye method). From the plasma volume and hematocrit reading, Stead and Ebert (5) calculated the red cell volume of splenectomized dogs before and after hemorrhage. The pre-hemorrhage red cell volume was from 21 to 34 per cent greater than that obtained from the sum of the post-hemorrhage red cell volume (plasma volume and hematocrit reading) and the volume of red cells removed. The latter determination was believed to approximate the value for the true red cell volume, because 75 per cent of the value for the red cell volume was determined by accurate measurement outside of the

body, and only 25 per cent was dependent upon the indirect measurement involving the assumption that the hematocrit determines the cell plasma ratio of all the blood in the body. They concluded that with an hematocrit reading of 50, the value for the red cell volume, as calculated from the plasma volume and hematocrit reading, was approximately 25 per cent higher than the true red cell volume. This study also showed that the value for total circulating hemoglobin obtained from the total blood volume (plasma volume and hematocrit reading) and hemoglobin concentration of blood removed from the body was approximately 25 per cent higher than the true total circulating hemoglobin. Studies in man have likewise shown that the red cell volume calculated from the plasma volume and hematocrit reading is falsely high (9).

Thus blood volume studies offer indirect evidence that the cell plasma ratio of blood removed from the body does not represent the actual cell plasma ratio of the entire blood. Smith, Arnold and Whipple (2) demonstrated in dogs that this discrepancy is not due to a difference between the hematocrit reading of blood from the extremities and that of blood from the viscera. This must also be true in man because the hematocrit reading of blood from the visceral organs would have to be considerably lower than that in the peripheral blood to account for the discrepancy in red cell volume. If this were true, there would be a sudden fall in the hematocrit reading of blood taken from the extremities when blood is shifted from the viscera by exercise or hemorrhage. This does not occur.

It was suggested by Smith, Arnold and Whipple (2) that the blood flowing in the minute vessels of the body might contain fewer red cells than the blood flowing in the large blood vessels. This idea was supported by the behavior of blood streaming in small vessels. In living tissue under the microscope the red cells are seen to occupy the center of the moving stream and to be surrounded by a clear area of plasma (6). This phenomenon is best seen in vessels of approximately 0.02 mm. in diameter. It can be seen in vessels as large as 0.1 mm. In the minute vessels the clear area of plasma is larger in comparison to total area of the vessel. Fåhræus (3) showed that, when blood is streaming in small tubes, it is impossible to obtain the true cell plasma ratio of the blood

contained in the small tube from the cell plasma ratio of the blood which flows from the cut end of the tube. The axial portion of the stream, which is rich in red cells, flows out of the cut end, while the slower moving peripheral layer of plasma remains in the tube. In his experiments, the hematocrit reading of blood contained in the tube was obtained by sealing the ends of the capillary tube and centrifuging it.

Direct proof that the blood within the minute vessels of the body is poorer in red cells than the blood flowing out of the minute vessels has been lacking. It is possible that there were unknown errors in the methods used to determine the plasma volume and that no thin blood was present in the body. The experiments reported here demonstrate that a portion of the blood usually contained in the minute vessels, in contrast to that normally flowing from these vessels, can be removed from the body and studied. This blood is richer in plasma and poorer in red cells than the blood normally flowing from these vessels. From work on splenectomized dogs, Stead and Ebert (5) concluded that the hemoglobin concentration of blood removed from the body is about 13 per cent higher than the hemoglobin concentration of the entire blood. The hemoglobin concentration of blood in the minute vessels must be considerably lower than that of the entire blood. In the experiments reported here, the hemoglobin concentration of the venous blood was 8 per cent greater than that of the blood from the small vessels. One would not expect the blood obtained by milking the forearm to have as low a cell plasma ratio as actually exists in the small vessels because it is mixed with some blood from the large vessels.

The fact that the cell plasma ratio of the blood in the minute vessels is lower than that of blood in the large vessels makes it impossible to determine accurately the red cell volume, the total circulating hemoglobin, or the total blood volume from the plasma volume, hematocrit reading, and hemoglobin concentration. The calculation of the total blood volume from the plasma volume and hematocrit reading has led to certain erroneous conclusions, particularly in the comparison of the total blood volume of anemic and normal subjects. In pernicious anemia, before liver therapy, the total blood volume, as measured from the plasma volume and hematocrit reading, is on the average